Two-stage MBR to reduce sludge production and total aeration volume

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TO REDUCE SLUDGE PRODUCTION
AND
TOTAL AERATION VOLUME

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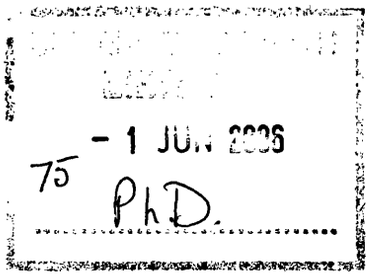
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ABSTRACT

Membrane bioreactor (MBR) has been gaining recognition in its use in wastewater treatment plants over the past decade. The use of a membrane bioreactor is very promising in ensuring a reduced excess sludge production from the process, as well as producing a high quality standard discharge. This thesis presents a new concept of the two-stage MBR, and work is focused on exploring the plausibility of the concept experimentally and economically. Kubota laboratory-scaled submerged membrane bioreactors have been developed for the experimental investigation of the different objectives in this research. The two-stage MBR concept offers the advantage of uncoupling the two different metabolic reactions in the biodegradation process, i.e. the removal of BOD and reduction of excess sludge production. Emphasis is made to ensure maximal BOD removal rate in the first stage, while the second stage has the object of reducing excess sludge production. Experimental observation over an operation period of a year shows high quality effluent being achieved within the first stage, while an overall sludge reduction of 50 – 60% is attainable for the two-stage MBR concept. Batch respirometry studies are also carried out in the attempt to determine the kinetic constants for both stages of MBR. Successful kinetic results are obtained for MBR 1, although the issue of higher initial food-to-microorganism (F/M or S_0/X_0) ratios employed in batch respirometry studies, compared to that found in MBR 1, raises questions on the application of the determined kinetic constants for MBR 1. Due to the complexity and lack of understanding of the processes such as cell lysis, cryptic growth, predation, etc. occurring in MBR 2, the batch respirometry studies has not been successful. Further work is needed in increasing the understanding of MBR 2. A steady state mass-balance model for the two-stage MBR is developed based on Monod growth kinetics to predict the performance of the MBR in terms of effluent quality and sludge production / yield. The model, solved with the kinetic constants determined from batch respirometry studies, successfully predicts the steady state performance for MBR 1, whereas the application of Monod kinetics is proved to be unfeasible for MBR 2. The economic assessment shows that the two-stage MBR still offers an annual savings due to the reduced sludge production despite the added capital investment cost of the second stage. Two-stage MBR may prove to be useful in the wastewater treatment industry in offering a reduction in the total sludge production and aeration volume if further understanding is achieved.

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"I sought the Lord, and he answered me; he delivered me from all my fears."

(Psalm 34:4)

The Lord appeared to us in the past, saying:
"I have loved you with an everlasting love; I have drawn you with loving-kindness"

(Jeremiah 31:3)

Nomenclature

A	Membrane area (m^2)
b_H	Endogenous decay rate of heterotrophic biomass (h^{-1} or d^{-1})
d	Annual depreciation ($/year)
D	Dilution rate (h^{-1})
DO	Dissolved oxygen concentration (mg O_2/l)
H	Height (mm)
HRT	Hydraulic residence time (h or d)
k_d	Endogenous respiration rate / biomass decay rate (h^{-1} or d^{-1})
K_S	Half-saturation substrate concentration (mg COD/l or mg BOD/l)
L	Length (mm)
μ	Specific growth rate of biomass (h^{-1})
μ_H	Maximum specific growth rate of heterotrophic biomass (h^{-1} or d^{-1})
μ_m	Maximum specific growth rate of biomass (h^{-1} or d^{-1})
n	Service life of MBR plant (yrs)
OUR	Oxygen uptake rate (mg O_2/l/h)
O_X	Cell COD (mg COD/ mg MLVSS)
P	Pressure (bar)
P_X	Waste sludge production (g MLVSS/d)
Q	Flowrate (l/min)
r or R^2	Correlation coefficient (in CurveExpert 1.3)
r_s	Substrate utilization rate (g COD/l/h)
r_x	Biomass growth rate (g MLSS/l/h)
S	Substrate concentration (mg COD/l or mg BOD/l)
S	Standard error (in CurveExpert 1.3)
S_S	Readily biodegradable substrate (mg COD/l or mg BOD/l)
SOUR	Specific oxygen uptake rate (mg O_2/g VSS/h)
SRT	sludge retention time (h or d)
t	Time (min or h or day)
T	Temperature (°C)
θ_C	Sludge age or sludge retention time (h or d)
V	Working volume (l)
V	Original value of MBR plant at start of the service-life period, completely installed and ready for use ($)
V_S	Salvage value of MBR plant at end of service life ($)
W	Width (mm)
X	Biomass concentration (g MLSS/l or g MLVSS/l)
X_H	Heterotrophic biomass (g MLSS/l)

X_I	Inert biomass (g MLSS/l)
X_S	Slowly biodegradable substrate (g COD/l or g BOD/l)
$Y_{X/S}$ or Y	Sludge yield (g MLSS/ g BOD removed or g MLSS/ g COD removed)

Subscript

0	Initial or inlet / feed
1	1st stage of MBR (MBR 1)
2	2nd stage of MBR (MBR 2)
2-stage	Two-stage MBR
C	Cell / biomass
O_2	Oxygen
P	Permeate
total	Total
W	Sludge wastage

Abbreviations

ADP	Adenosine 5'-diphosphate
ADWF	Average dry weather flow
APHA	American Public Health Association
AS	Activated sludge
ATP	Adenosine 5'-triphosphate
BAF	Biological aerated filter
BOD	Biochemical oxygen demand
BOD_5	5-day biochemical oxygen demand
CFU	Colony forming unit
CNP	4-chloro-2-nitrophenol
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
*d*NP	2,4-dinitrophenol
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EC	European Communities
EMP	Embden-Meyerhof-Parnas (pathway)
ENR	Engineering News-Record
EPA	Environmental Protection Agency

F/M	Food-to-microorganism ratio
HRT	Hydraulic residence time
IAWQ	International Association on Water Quality
LMH	Litre/m^2.h (nit for flux)
LPM	Litre/min (unit for air flowrate)
MBR	Membrane bioreactor
MBR 1	1st stage of the two-stage membrane bioreactor
MBR 2	2nd stage of the two-stage membrane bioreactor
MF	Microfiltration
MGD	Mega gallon/day (unit for flowrate)
MLSS	Mixed liquor suspended solids (or sometimes abbreviated to SS)
MLVSS	Mixed liquor volatile suspended solids (or sometimes abbreviated to VSS)
NDIR	Non-dispersive infrared gas analyser
nP2	Permeate of the new MBR 2
O&M	Operating and maintenance (cost)
OECD	Organisation for Economic Cooperation and Development
OSA	Oxic-settling-anaerobic (process)
OUR	Oxygen uptake rate
P1	Permeate of MBR 1
P2	Permeate of the original MBR 2
PE	Population equivalence
PHA	Poly-β-hydroxyalkanoate
PHB	Poly-β-hydroxybutyrate
*p*NP	Para-nitrophenol
PWF	Pure water flux
PYG	Peptone, Yeast and Glucose (supplement for amoeba culture)
RNA	Ribonucleic acid
SA	Sample analysis
SBR	Sequencing batch reactor
SMBR	Submerged membrane bioreactor
S_0/X_0	Initial substrate concentration to initial biomass concentration ratio (similar to F/M), but the term is used for batch respirometry studies
SOUR	Specific oxygen uptake rate
SRT	Sludge residence time or solids retention time or sludge age
SVI	Sludge volume index
TCA	Tricarboxylic acid cycle
TCP	Trichlorophenol
TCS	3,3',4',5-tetrachlorosalicylanilide
THMs	Trihalomethanes
TMP	Transmembrane pressure

TN	Total nitrogen
TNT	Test'N Tube (Hach's tests for nitrogen concentrations)
TOC	Total organic carbon
USEPA	United States Environmental Protection Agency
VSP	Volumetric sludge production
ΔCOD	Substrate utilization (COD)
ΔCOD_{cell}	Cell / biomass synthesis (COD)
ΔX	Amount of cell / biomass produced

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Chapter 1: Introduction

Scope of Chapter 1

This chapter will introduce the fundamental concepts and the state-of-the-art developments of membrane bioreactors. An analysis of the respective merits of employing a membrane bioreactor in the wastewater treatment process, relative to the conventional activated sludge process (AS), will be presented.

1.1 Background to the Project

The primary aim of domestic sewage treatment is to safely dispose of treated wastewater without causing a pollution or environmental problem (Burns and Slater, 1982) and, secondly, to conserve natural resources by allowing the water to be recycled to industry, agriculture or the home. This aim is achieved by three methods:

1) the reduction in BOD
2) the reduction in the numbers of pathogenic microorganisms
3) the reduction in the levels of inorganic nutrients, such as phosphorus and nitrogen, which can lead to eutrophication problems in standing waters

1.2 Activated Sludge (AS) Process

Activated sludge has long been established as the most widely used biological wastewater treatment process for both domestic and industrial wastewater. The concept was developed into a full-scale unit process in 1913 by Arden and Lockett, at the Davyhulme Treatment Works in Manchester. The process employs a highly bacteriologically active sludge (termed 'activated sludge') to utilise the organic matter, which in turn is being converted into stable respiration end products (CO_2, NO_3, SO_4 and PO_4), water and the biosynthesis of new microorganisms under aerobic conditions. With unlimited food and oxygen supply, high rates of microbial growth and respiration can be promoted.

The activated sludge is distinctive in its two-phase process, aeration and sludge settlement. Originally designed as a batch process known as the fill and draw method with both aeration and sludge settlement occurring in the same tank, now a continuous system with two separate units is used. The first tank consists of the aeration device,

where air is added either by surface agitation or via diffusers using compressed air. The aeration serves two purposes: to provide the oxygen required by the aerobic microorganism for respiration and to maintain the microbial floc in a continuous state of agitated suspension, ensuring maximum contact between the surface of the floc and the wastewater (Gray, 1999). This continuous mixing action is important as it not only ensures adequate food, but also a maximum oxygen concentration gradient to enhance mass transfer and to help disperse metabolic end products from within the floc. Settled wastewater after the primary settling tank enters the aeration tank and displaces the mixed liquor (the mixture of wastewater and microbial biomass) into the second sedimentation tank. In this settling tank, flocculated biomass settles by gravity to the bottom of the tank and results in a virtually solids free, clarified effluent, which is subsequently discharged. Most of the settled sludge is then returned to the aeration tank as an inoculum to ensure sufficient microbial population in the tank for the biodegradation of organic wastes.

The advantages of an AS process are its simplicity and robustness in its design and maintenance. However, the rate-limiting step lies in the settling stage, whereby the separation of sludge floc from the treated effluent is by gravitational sedimentation. The separation of biomass is promoted by excreted bacterial exopolysaccharides that bind a mixed microflora into flocs to settle. Good separation is, therefore, highly dependent on the flocculating ability and settleability of the aggregated mixed microbial flocs. Consequently, the AS process is restricted to operating at a low biomass concentration (2,000 – 5,000 mg/l) resulting in large reactor volumes and low conversion rates. Due to the increasing demands to comply with stricter discharge consents, there is considerable impetus towards developing new technologies to overcome the limitations of the AS process.

1.3 Respective Merits of Membrane Bioreactor (MBR) Technology

The concept of the membrane bioreactor (MBR) is a hybrid system of the existing biological AS treatment process with the integration of a membrane module as the separating/filtering agent. The use of the membrane to replace the secondary sedimentation stage overcomes the operating constraints of the AS process and greatly improves the process efficiency in meeting heavier wastewater loading rates and the increasingly stringent discharge standards (Chang and Judd, 2002). There has

been, therefore, a rapid development and widespread application of the innovative membrane bioreactor technology, owing to its advantages:

- Uncoupling of the two important process parameters, hydraulic retention time (HRT) and solid retention time (SRT), allowing the absolute control of SRT, which makes operation at high concentrations of mixed-liquor suspended solids (MLSS) possible (up to 30 g MLSS/l)
- Perfect and complete retention of biomass, hence overcoming the problem of washout
- The membrane offers a physical barrier to the organisms that is unaffected by the influent quality, thus ensuring a high quality disinfected discharge effluent free of bacteria, pathogens and viruses. Reductions in bacteria and viruses of 4 – 8 log have been reported (Kolega *et al.*, 1991; Chiemchaisri *et al.*, 1992; Jefferson *et al.*, 2000; Gander *et al.*, 2000a)
- Low footprint, compact and robust with a low maintenance requirement
- Easy automatic control of the whole system. Complete nitrification in a MBR is observed with a HRT as low as 7.5 hours (Urbain *et al.*, 1998)
- Maximal BOD removal rate, as high organic loading rate is possible with the dissociation of HRT and SRT
- The problems with the presence of filaments or bulking sludge, during hydraulic peak loads have been overcome by the replacement of the rate-determining, sedimentation stage
- The system could be operated with a higher sludge age which promotes autolysis and endogenous respiration, leading to a lower excess sludge production
- Increase in contact time of large macromolecules with the biomass, allowing effective treatment of low biodegradable products
- The air recirculation also guarantees no anoxic/septic zone within the tank, thus resulting in minimal odour release (no sulfite produced)

Realization of new process engineering in wastewater treatment industry is made possible with the application of the MBR technology. Figure 1.1 shows how a membrane bioreactor replaces the individual treatment units of a wastewater treatment plant. Due to the high concentration of suspended solids that can be retained in the membrane bioreactor, the volume of the tank is considerably reduced. The

comparison also shows less process stages for the whole process employing the MBR technology, thus introducing the possible space-saving conception (Engelhardt *et al.*, 1998). Conclusively, the use of a membrane bioreactor offers a compact system with unrivalled effluent quality.

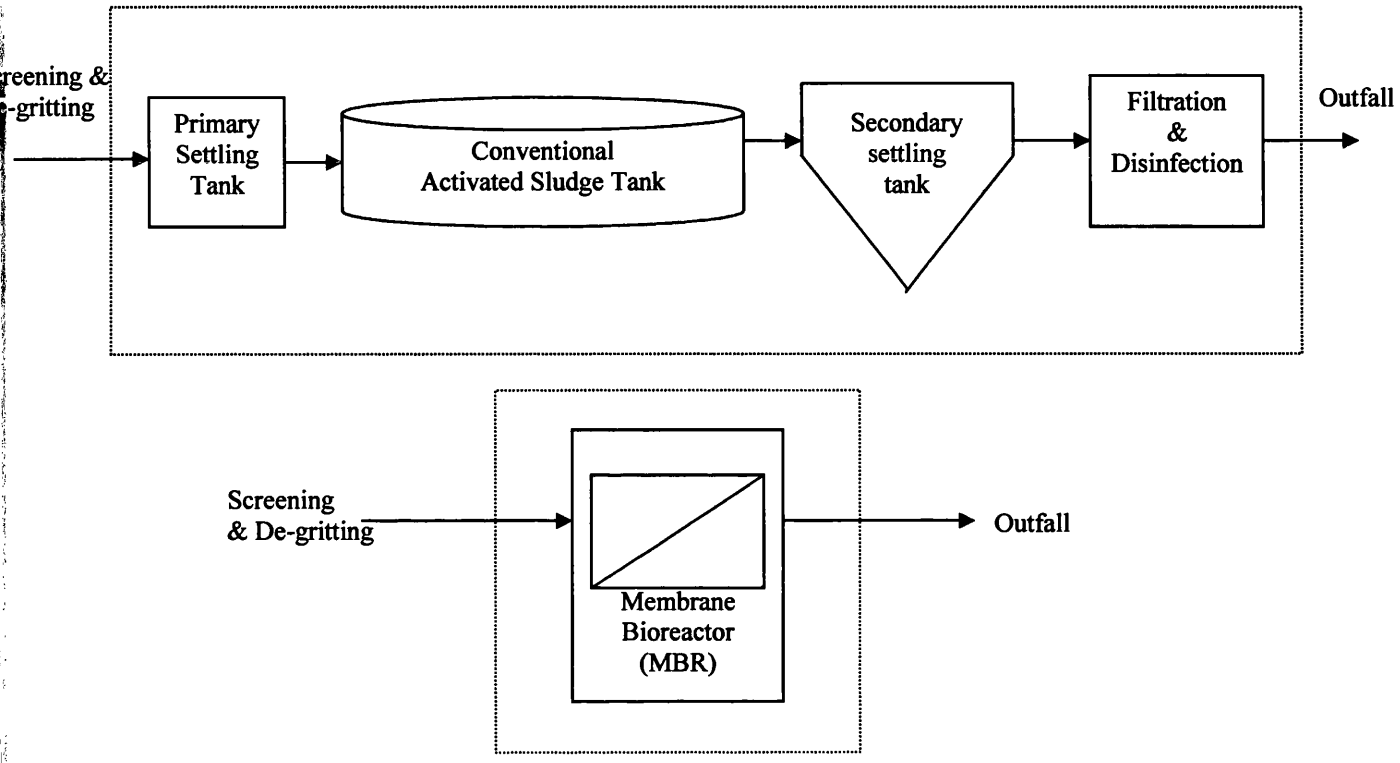


Figure 1.1: Comparison of conventional wastewater treatment with the integrated MBR technology process

1.4 Limitations of the MBR technology

One of the major limitations of the membrane bioreactor technology is the fouling phenomenon. This occurs when a layer of foulant is gradually formed on the surface of the membrane filters, increasing the membrane hydraulic resistance. The performance of the membrane will be reduced and eventually no flux will be flowing through the membrane. This however can be reduced by maintaining turbulent conditions, operating at sub-critical flux and/or by using a suitable fouling resistant membrane material (Gander *et al.*, 2000b). The MBR technology has its constraints in the economics as the membranes are expensive and the aeration cost contributes to the major operating cost. However, recent developments and increased production of

membranes by the manufacturing companies has led to a decrease in the membrane fabrication cost worldwide.

1.5 Current State of Membrane Bioreactor (MBR) Technology

There are two principal configurations for the membrane bioreactor system, i.e. i) a sidestream cross-flow membrane unit, and ii) a submerged/immersed membrane unit (refer to Figures 1.2(a) and (b)). The sidestream configuration consists of a membrane module in an external recirculation loop, where the concentrated MLSS is recycled back to the biological reactor. The operating mode of the sidestream configuration at higher Reynolds numbers offers better control of concentration polarisation than the submerged configuration (Gander *et al.*, 1999). However, there are problems arising from this type of configuration:

- high power consumption cost due to the pumping of a high viscosity return sludge stream, between 2 to 10 kWh/m^3 of water produced (Coté *et al.*, 1998)
- high shear stresses in the recirculation pumps leads to the destruction of bioflocs and hence loss of biological activities (Seyfried and Brockman, 1995)
- the system requires sophisticated maintenance and operational control in terms of chemical cleaning and back-washing operation due to high flux and high pressure operation of the external membrane unit (Ishida *et al.*, 1993)

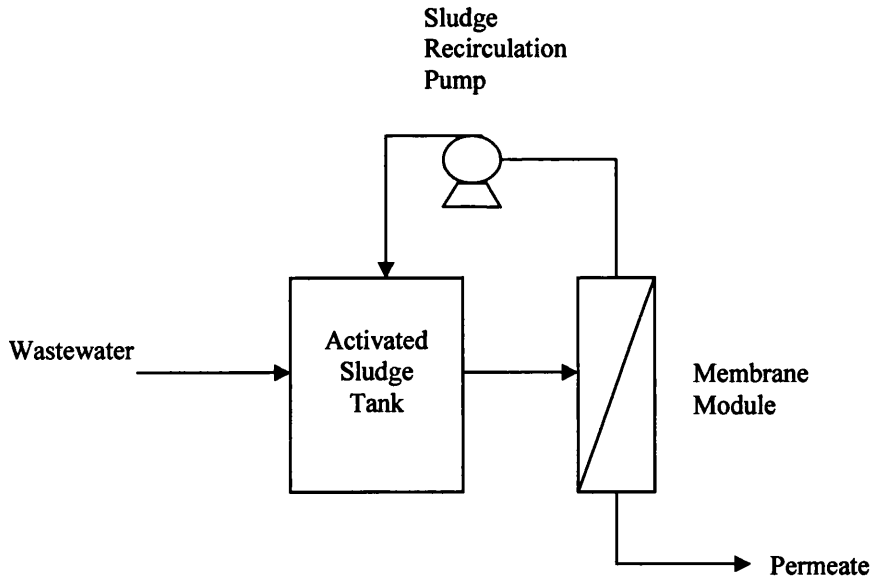


Figure 1.2(a): Sidestream Cross-flow Membrane Bioreactor Unit.

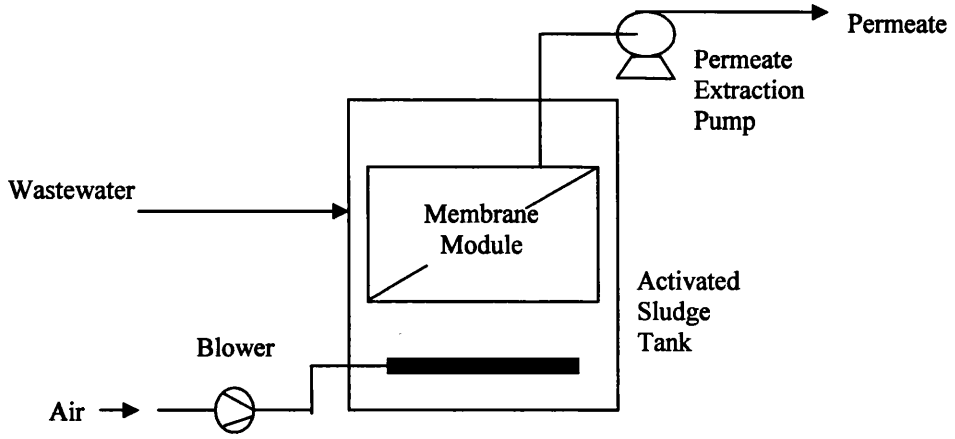


Figure 1.2(b): Submerged / Immersed Membrane Bioreactor Unit.

With regards to the above constraints with the sidestream MBR configuration, this leads to a new development in the immersed membrane bioreactor configuration. In this configuration, the membranes are directly submerged into the activated sludge biological tank and the treated permeate is extracted by a suction pump or by gravity. This configuration results in remarkable savings in energy costs. Coté (2000) reported the energy consumption including pumping and aeration to be between 0.3 to 0.6 kWh/m^3 treated and this quantum gain in terms of energy is the primary reason for the success of immersed membranes in North America for a wide range of applications. Therefore, the immersed MBR configuration has the potential to be applied to small wastewater treatment plants. It also enables the upgrading and extension of an existing plant with the integration of the membrane module into the activated sludge tank.

One of the main features of the configuration is its self-cleaning membrane mechanism, in which the sweeping action of the rising coarse bubbles prevent fouling and the build up of a cake layer on the membrane surface, hence reducing the need for chemical cleaning and maintenance. Gander *et al.* (2000b) commented on the differences in the cleaning requirements for the two MBR configurations. As the permeate flux is relatively lower in a submerged MBR compared to a sidestream configuration, it can be maintained for extended periods without decline. They reported that a submerged plate and frame MBR configuration only employed a twice-yearly membrane clean with a 0.1% sodium hypochlorite (NaOCl) solution. On the other hand, the flux and concomitant fouling rate is much higher in a sidestream

system. Therefore, sidestream systems often require more frequent and rather more aggressive cleaning achieved by back-washing (reversal of permeate flow for a short time at filtration pressure) or chemical cleaning. One such system based on a ceramic membrane required weekly cleaning using 5.25% NaOCl heated to 60 – 80°C along with concentrated nitric acid, and the cleaning cycle lasted about 2 hour. Also, such cleaning conditions demand a very resilient membrane material.

Table 1.1 summarises the advantages and disadvantages of the different MBR configurations.

Table 1.1: Advantages and disadvantages of the different MBR configurations (Till and Mallia, 2001).

Side-stream MBR	Submerged MBR
Low aeration costs (~ 20%)	High aeration costs (~90%)
High pumping costs (60 – 80%)	Very low liquid pumping costs (higher if suction pump is used ~ 28%)
Higher flux (smaller footprint)	Lower flux (larger footprint)
More frequent cleaning required	Less frequent cleaning required
Higher operating cost	Lower operating costs
Lower capital costs	Higher capital costs

1.6 Commercial MBR systems

Kubota (Japan) and Zenon (Canada) are the two main suppliers of commercial MBR systems for wastewater treatment (Till and Mallia, 2001). Other suppliers are Degremont (France), Membratek (South Africa), Orelis/Mitsui (Japan), US Filter (USA) and Wehrle Werk (Germany). The following section compares the two main MBR systems available in the market now.

1.6.1 Kubota Submerged Membrane Bioreactor (SMBR) Technology

The Kubota submerged membrane effluent treatment process is one of the few available membrane bioreactor systems that have seen commercial application (Churchouse, 1997). It was first developed by Kubota Corporation Environmental Plant Division in Japan to meet the increasing demand for compact, low footprint, and

a high quality effluent with re-use capabilities (Ishida *et al.*, 1993). It is essentially a high MLSS activated sludge process, in the range of 15-20 g/l MLSS where the Kubota membrane treatment units are submerged within the activated sludge tank.

The Kubota MBR is of plate and frame configuration. The Kubota membrane is a flat sheet membrane made of polyolefin and composed of a non-woven fibrous support material coated with a porous selective layer nominal pore size of 0.4 μm (Davies *et al.*, 1998). The membrane is welded ultrasonically onto the outer surfaces of both sides of an injection moulded plastic flat plate. The typical membrane cartridge (Type 510) has dimensions of 1.0 m (H) × 0.49 m (W) × 6 mm thick. The permeate passes through to the interior of each membrane to an outlet nipple cast into the top of the support plate. Each cartridge has an effective filtration area of $0.8m^2$.

The standard Kubota unit has a glass fibre reinforced plastic casting and consists of two sections (Churchouse, 1997). The upper section contains up to 150 individual membrane cartridges, arranged vertically at a gap of 7 mm between the panels, and each connected to a permeate collection manifold. The lower section underneath the membrane modules is the coarse bubble air diffuser system, in which the coarse bubbles generate an uplifting cross-flow velocity across the membrane surfaces. The air-water mixture maintains an upward cross flow over the membrane surface of approximately 0.5 m/s, providing a continuous cleaning effect and thus minimising fouling of the membranes. The aeration system also serves the function of supplying the oxygen needed for the aerobic conversion process. The minimum air requirement is 10 L/min for each membrane cartridge. Davies *et al.* (1998) reported a constant air supply of 142 m^3/h to 200 panels of membrane cartridges (11.8 L/min for each membrane cartridge) and Churchouse (1997) reported a constant air flow of 220 m^3/h to 300 panels of membrane cartridges (12.2 L/min for each membrane cartridge).

The Kubota system operates by gravity, with a liquid head of 1 – 1.5 m above the membranes sufficient to drive permeate through the membranes (Till and Mallia, 2001). Grit removal and fine (2-3mm) screening are pre-requisites prior to the MBR. The membrane flux for the Kubota system is approximately $20L/m^2.h$ (submerged system at a TMP of ~ 0.1bar). Chemical cleaning of the membranes is required every

3-6 months using sodium hypochlorite and oxalic acid. Cleaning requires 3 L of chemical solution per cartridge and the cleaning cycle takes up to 2 hours.

Kubota has a reference list of over 600 plants treating domestic and industrial wastewater, with most of the sites located in Japan. The Kubota plants range in size from systems to treat the equivalent of individual households to the 23,000 PE (5,800m^3/d ADWF) plant at Swanage in the south of England.

1.6.2 Zenon MBR system

Zenon markets the ZenoGem system, based on the ZeeWeed membrane, which is a hollow fibre with an external diameter of 1.9 mm and a nominal pore size of 0.4 μm. The membrane module consists of hundreds of membrane fibres oriented vertically between two headers. The hollow fibres are slightly longer than the distance between the top and bottom headers and this allows them to move when aerated. The filtered effluent / permeate passes into the centre of the fibre and is extracted from both ends. The ZeeWeed® 500 module is 2.0m (H) x 0.7m (W) x 0.2m thick with 46 m^2 of filtration surface area. Cassettes are made up of 8 modules each. Air is supplied to the system by a combination of coarse bubble aerators integrated into the bottom header of modules, to gently agitate and scour the membrane fibres and to keep the tank contents mixed, and by fine bubble aeration to supply the balance of the total biological oxygen demand.

The ZenoGem MBR system could support a MLSS concentration of 15 – 20 g/l. The typical biological design parameters include a F/M ratio below 0.2 kg BOD_5/ kg MLSS.d and a volumetric loading of 1.8 – 7.5 kg BOD_5/m^3.d. Typical HRT and SRT are above 2 hours and 15 days. The operational flux is between 15 – 25 LMH at a TMP of ~ 0.5 bar).

Cleaning of the membranes is provided by automatic pulses of backwashing with stored permeate and periodic in-situ membrane cleaning with a hypochlorite solution or other chemicals.

Zenon has a reference list of over 150 plants treating domestic and industrial wastewater. Zenon also has applications in the water treatment industry. Zenon will supply its ZeeWeed® membranes to a new 72 MGD (273,000 m^3/day) drinking water plant to be built in Singapore, which will be one of the world's largest membrane-based water treatment plants.

1.7 Trends in the Development of MBR Technology

Since the year of 1992, submerged membrane bioreactor technology has seen commercial application with an increased scale of more than 100 fold. The reason for the rapid development in the field of membrane bioreactor technology is a result of the approximate halving of the capital cost and an effective ten fold reduction in the projected operating costs compared to 8 years ago (Churchouse and Wildgoose, 1999). Table 1.2 shows a comparison of the reasons of the lack of application of this technology 5 years ago in the wastewater plant industry and the increase both in the number and diversity of the applications now.

Kubota submerged membrane bioreactor process has developed rapidly over the last few years and there is over 600 plants currently in application worldwide. The largest Kubota submerged MBR treatment plant in the world, operated by Wessex Water, has been commissioned in July 2000 on the picturesque south coast of Dorset. It has a design capacity to treat effluent from a summer population of 28,000. It receives a full incoming flow of 12,700 m^3/d. The vast application of the Kubota process is due to the overall significant reduction in the capital and operational costs. The major developments allowing the cost reduction since 1992 includes the doubling of design flux rate with the use of gravity flow, the scale up of membrane manufacturing and also an increase of projected membrane life span. These improvements have led to a subsequent reduction in plant size and power consumption, a decrease in the cost of membrane panels replacement by 15 times and simplified plant design and operation. In addition, the decrease in the absolute cost of membrane units provided to large-scale installations, with current prices in the region of £20 per square meter for the largest installations, has led to a steady increase in the application of the membrane bioreactor technology for wastewater treatment industry. This technology will no doubt prove itself promising in competing with the conventional wastewater treatment

processes in the near future in terms of meeting the tighter effluent standards and restrictions of landscape.

Table 1.2: MBR Technology in year 1995 and year 2000.

Year 1995 Disadvantages of MBR Technology	Year 2000 Benefits of MBR Technology
Unproven and complex technology	Reliable and simple operation
Small, lab scale research	Full scale industrial application for flows in excess of 10,000 m^3/d
Unknown maintenance and labour requirement	Reduced maintenance and labour requirement
High membrane failure rate	Low membrane failure rate, < 0.3% pa over 5 years
High process operational cost	Substantial reduction in overall process cost due to increase in scale and usage
High membrane replacement cost	Projected membrane lifespan has increased from 3 to 8 years – reduced membrane replacement cost
No requirement in meeting stringent discharge consent	Introduction of stricter legislation on discharge effluent quality

1.8 Concept of a 2-stage Membrane Bioreactor (MBR)

In a wastewater treatment plant, the production of sludge is proportional to the volume of the waste input loading. Therefore, an effective treatment process aims to achieve the highest possible removal rate of pollutant organics at the minimum period of time, hence reducing the volume of reactor required. The reduction in volume also leads to a decrease in the footprint of the treatment plant and therefore a reduction in capital cost. On the other hand, the generated excess sludge imposes a burden with its expensive handling (drying, disposal, etc.) cost. In a single reactor, the mechanism of maximal biodegradation of pollutants will be contradicted with the aim of minimal excess sludge production. Therefore, in order to exercise cost-effectiveness while still complying with the discharge consent, a compromise has to be made between the two

objectives in a single treatment unit. However, instead of finding the middle ground, the concept of a 2-stage MBR system is proposed for the first time to accomplish both of the main concerns in wastewater treatment process.

The aim of this project is to look at the possible benefits of operating the MBR system in two stages in terms of minimising the aeration volume and the excess sludge production. The concept of the 2-stage MBR system uncouples the two contradicting mechanisms of maximum bioconversion and reduced sludge production. The first stage will be focused on the removal of BOD at the highest possible rate, and hence in a smaller volume reactor using a high food-to-microorganism (F/M) ratio. The food refers to the feed input or organic loading to the system, while the microorganism refers to the biomass concentration in the bioreactors. For MBR systems, the dissociation of the hydraulic retention time (HRT) from the sludge retention time (SRT) offers absolute control of SRT, which makes it possible for the second stage to be operated at a high biomass concentration. The concentration of the sludge in MBR 2 can be achieved by operating on a high SRT, i.e. less sludge wastage. With MBR 2 only receiving wasted sludge from MBR 1 as a source of feed, it will focus on aerobically reducing the net sludge production via endogenous metabolism. Maintaining a low food micro-organism (F/M) ratio in the bioreactor will result in minimum sludge production and the low feed rate means a smaller plant size for the second stage.

1.9 Conclusions

Submerged membrane bioreactor is a promising technology that is gaining vast applications in the wastewater treatment industry. This project will focus on the concept of two-stage MBR to reduce the excess sludge production and hence the total operational volume of the unit. Further sludge reduction strategies will be presented in Chapter 2 Literature Review – Sludge Reduction Strategies.

Chapter 2: Literature Review - Sludge Reduction Strategies

Scope of Chapter 2

This chapter discusses the economical and environmental challenges posed by the increasing demand of excess sludge production. It also gives a review on the sludge reduction strategies within the existing literature. Possible mechanisms and processes occurring in the second stage of the MBR are also reviewed to give an understanding of the physiology of the microorganisms.

2.1 Sludge Disposal Issue

A wastewater treatment process is basically a production unit of biosolids with the treated water as the non-profitable by-product (Lowe, 2000). As the demand to meet tighter emission standards increases, and with the increasing wastewater loadings, the wastewater treatment works carry with them a penalty for increased sludge production. With the introduction of the EC Urban Waste Water Treatment Directive (91/271/EEC), secondary treatment is now mandatory for all inland towns and for those discharging to estuaries with a population equivalent (PE) in excess of 2000, and for towns discharging to coastal waters with a PE > 10,000 (Gray, 1999). The State of the Environment report, published by Ireland's Environmental Protection Agency (EPA) in April 1996 (Source: http://www.ncte.ie/environ/water.htm), has indicated that significant upgrading of sewage treatment facilities will be necessary in order to meet the requirements of Directive 91/271/EEC concerning urban waste water treatment, particularly with regard to discharges to tidal waters. This will result in at least 50% more sludge being produced by the end of 2005 (Mayhew, 1999). Therefore, there is immense pressure for the wastewater industry to strike a fine balance between water quality and sludge production.

Sludge production and disposal are entering a period of dramatic change, particularly as an outcome of increasing environmental awareness and economical impact. Previous sludge disposal options listed in order of EC preference of use (Hall, 1996; Matthews, 1996) include recycling to agricultural land, land restoration, novel uses, incineration, landfill and coastal dumping. However, there are increasing difficulties

in sludge disposal to all the established outlets, which in the case of sea disposal have become illegal by 1 January 1999.

The biosolids produced is being regarded as a "risk" product to the environment under UK and European regulations, in terms of the metal content and the nuisance generated by the odour released. EC Directive 86/278/EEC has focused on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. The UK "Safe Sludge Matrix" has recently introduced the concept of an "Advanced Treated Sludge" which qualifies the use of sewage sludge for agricultural land depending upon the level of treatment given. There is also considerable concern in terms of the potential risk of sewage sludge as a source for the release of pathogens into the environment, particularly into the food chain. The US Environmental Protection Agency (EPA) generated the 503 rule that deals with the pathogen issue and sets standards for a "Class A Sludge". This particular disposal route may not be the simplest or cheapest due to the pre-requisite of the sludge being treated before disposal onto the land.

Biomass production is an important economic factor because the sludge generated is a secondary waste that must be disposed of in an environmentally sound and cost-effective manner. It poses as a serious problem because the treatment of the excess sludge may account for 25 – 65% of the total plant operation cost (Horan, 1990; Zhao and Kugel, 1997). Hence, there is a positive momentum for developing strategies to minimise the biosolids production in existing plants or for seeking alternatives of new innovative technologies that guarantee lower sludge production.

The submerged membrane bioreactor offers a solution to achieving the minimisation of excess sludge production while meeting the required discharge quality. Ishida, H. *et al.* (1993) reported a 30% reduction of sludge production compared to AS process and improved running costs with the Kubota submerged membrane effluent treatment process for domestic sewage waste in Japan. In terms of the quality of the treated water, the system ensures a standard higher than demanded by various discharge regulations.

2.2 Ecosystem Manipulation by Inducing Protozoal Grazing

2.2.1 Microbial Population within the Wastewater Treatment Process

The biological wastewater treatment processes employ a mixed microbial population to simulate a real-life ecological system in order to decompose and biodegrade the organic pollutants present in the influent feed. Hence, it could therefore be considered as an artificial ecosystem. The microorganisms in the biological wastewater treatment system obtain the source of energy through the mineralization of the organic pollutants for maintenance, catabolism and anabolism (biosynthesis of cells). Mineralization is the oxidation of organic substances into CO_2, H_2O and N- and P-compounds.

In general, the diverse microbial community consists of bacteria, protozoa, metazoa, larvae of insects and arachnida (Curds and Hawkes, 1975). The organic pollutants contribute to the food source for saprophytic bacteria, which forms the basic trophic level, and is in turn consumed by predators such as protozoa and metazoa that makes up a link of food chain. Energy is passed through the trophic levels of the food chain. As a large amount of energy is dissipated during respiration, (about 90% of the available chemical energy is lost each time energy is transferred from one trophic level to the next higher one), the biomass of a lower trophic level is always, in a balanced community, greater than that succeeding it. Elton (1935) represents this successive links of food chain as a pyramid of numbers. Each horizontal section represents a trophic level, and the organisms occupying the same trophic level are competing for a common food supply. This is known as a horizontal relationship. The successive levels are linked by a predator-prey relationship, which is also known as a vertical relationship. At the base of the pyramid (first trophic level) there is always a green plant or other autotrophs which trap energy, almost always from sunlight, and produces food sources for the other levels (Allaby, 1979). At the second trophic level is a herbivore (primary consumer). At subsequent levels are smaller, then larger carnivores (secondary consumers). The Eltonian pyramid numbers is shown below in Figure 2.1.

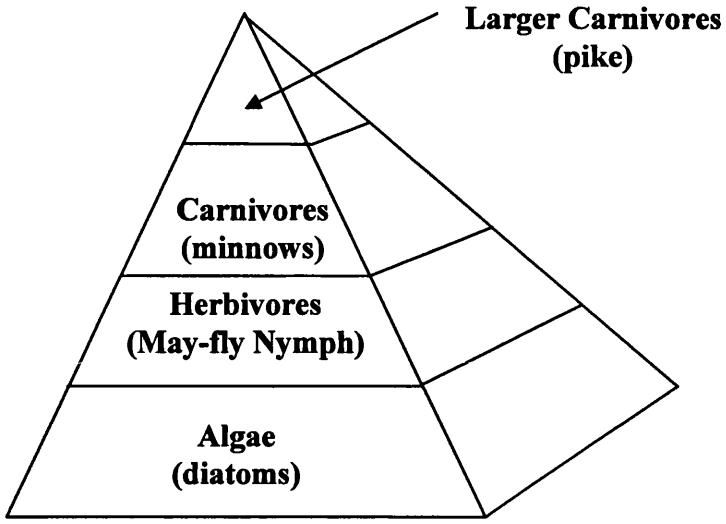


Figure 2.1: Populations in a stream community represented as an Eltonian pyramid of numbers (Hawkes, 1963)

The presence of protozoa in biological wastewater treatment indicates a healthy population of the saprophytic bacteria as it demonstrates the existence of a food chain. The role of protozoa and metazoa in conventional aerobic processes is to consume the dispersed bacteria and to induce flocculation in order to produce a clear effluent of good quality (Curds, 1992; Ratsak *et al.*, 1996). Hence, the presence of specific species of protozoa also serves as an indicator function of the quality of the effluent treated. Recent research (Ratsak *et al.*,1994; Lee and Welander, 1996; Rensink and Rulkens, 1997) shows that protozoal grazing on bacteria leads to a considerable biomass reduction. The reduced biomass production is due to a further degree of mineralization of the sludge by the protozoa in which the biomass is converted into new biomass, water and CO_2 (Hawkes, 1963). Due to inefficient biomass conversion, energy is lost during the energy transfer from biomass to protozoa (Berninger, 1990). Therefore, the possibility of sludge reduction in wastewater treatment processes is reviewed through the manipulation of the ecosystem.

2.2.2 Predation Phenomenon in the 2-Stage MBR

In conventional aerobic processes, the presence of predators suppresses the growth of dispersed bacteria and favours the growth of floc or film forming bacteria, which are more protected against predation (Gude, 1979). This selection pressure can be overcome by the use of a two-stage system, in which the first stage induces the growth of dispersed bacteria, which is later consumed by the predators grown in the

second stage. The first stage is operated as a completely mixed reactor without biomass retention and at a short solids retention time (SRT) to prevent the growth of predators and to induce the growth of fast-growing, dispersed bacteria. The second stage is operated at a long sludge age to promote the growth of bacterivores such as protozoa and metazoa. Ratsal *et al.* (1994) observed a reduction of biomass production by 12-43 % in a serial two-stage laboratory chemostat system with a pure culture of bacteria. Lee and Welander (1996) also used a two-stage configuration, with the second stage designed as a biofilm system, and found that a sludge yield of 0.05-0.17 g SS (g $COD_{removed}$)$^{-1}$ was obtained from treating a synthetic wastewater, i.e. a yield around 30-50% of those typically obtained in conventional aerobic treatment processes. Regarding the use of metazoa, Rensink and Rulkens (1997) found a sludge reduction from 0.40 to 0.15 g SS (g $COD_{removed}$)$^{-1}$ with the inoculation of *Tubificidae* worms in a pilot activated sludge system treating settled domestic wastewater.

The two-stage system or the inoculation of metazoa has a clear potential for a minimised sludge production; however there are unresolved problems such as the increased dissolved nitrogen and phosphate concentration in the treated effluent and disturbance of the nitrification capacity by the increased predatory activity in the second stage. Increased mineralization of biomass by predators also results in a considerable increase in oxygen consumption. Thus, the costs for increased aeration should be weighed against the benefits of a lower sludge yield. Apart from these, the formation of bacterial aggregates and fast-growing filaments, such as *sphaerotilus natan*, may give rise to potential problems related to biomass reduction in the 2nd stage as these growth forms are not as easily consumed by filter-feeding organisms, as are the dispersed bacteria. Hence, an approach to these would be to optimise the process in such a way that ensures the predominance of dispersed bacteria in the 1st stage.

2.3 Optimization of Process Parameters

Another approach taken to reduce the sludge production is to optimise the process parameters in the system. Laboratory scale experiments show that excess sludge production is affected by the sludge loading and oxygen concentration. It is well known that excess sludge production decreases with a decreased sludge loading rate

or with an increased solids retention time (SRT) in aerobic treatment processes (Henze *et al.*, 1987; Saunamaki, 1988). Abbassi *et al.* (1999) observed that the reduction of excess sludge production could be achieved by raising the concentration of dissolved oxygen in the mixed liquor. Canales *et al.* (1994) studied the effects of temperature on the maintenance and cryptic growth phenomena of *Pseudomonas fluorescens* culture using a MBR. With a thermal treatment in line on a recirculation loop on the MBR, biomass growth on these cell lysis products was achieved to characterise cryptic growth and its impact on sludge production.

2.3.1 Effects of Dissolved Oxygen Concentration

In aerobic treatment processes, dissolved oxygen serves as a hydrogen or electron acceptor and becomes reduced to water. Hence, it is an exceedingly important factor for the oxidation of organic pollutants by the aerobic microbial population. Liu and Tay (2001) commented on the importance of the supply of dissolved oxygen playing a limiting role for further increasing loading rates of treatment facility in full scale activated sludge process. McWhirter (1978) demonstrated a reduction in the growth yield of 54% in purified oxygenation activated sludge process compared to conventional air-activated sludge system even at high sludge loading rates. Boon and Burgess (1974) also found a much lower observed sludge yield in the pure oxygen system, 60% of that in the air system. In a laboratory-scale conventional activated sludge reactor, Abbassi *et al.* (1999) reported a decrease in the excess sludge production from 0.28 to 0.2 mg MLSS/ mg BOD_5 as the reactor DO was increased from 1.8 to 6.0 mg/L. They proposed that the optimum utilisation of cell hydrolysis within the bacterial floc matrix could be achieved by increasing the dissolved oxygen concentration, leading to a reduction in excess sludge production.

The relation between a reduced sludge production with high DO operation is not completely understood. McWhirter (1978) postulated that the high DO concentration would produce a higher level of active biomass, and hence, a lower true sludge loading rate. The lower true sludge loading rate will lead to a relatively lower sludge production rate at the same apparent measured value of the sludge loading rate, as compared to a low DO system. On the other hand, Ozoguz (1996) proposed different possible nutrient profiles in a floc matrix, depending on mass transfer resistances, in order to understand the oxygen utilisation mechanism in the bacterial floc matrix in an

aerobic mixed culture. When the floc was subjected to substrate limitation, three main, different regions can be recognised (Figure 2.2):

1. Region A of the floc is supplied with both substrate and oxygen and hence, the degradation of the bulk substrate S_F takes place in this part of the floc. The microbiological oxidation leads to a very large reduction in the substrate and oxygen concentrations.
2. Region B appears as the result of the floc exposure to a high oxygen concentration. As only oxygen is available, the content of dead cells and the available nitrogen will be degraded in this part of the floc. Degradation of cell contents increases as the size of this region increases, resulting in a reduction of the excess sludge produced.
3. An anaerobic Region C resides in the core of the floc, where oxygen is no longer available. The micro-organisms in this part are inactive and do not contribute to the degradation process. However, the hydrolysis of the cells continues and the lysing cells generate a new substrate, known as the secondary substrate.

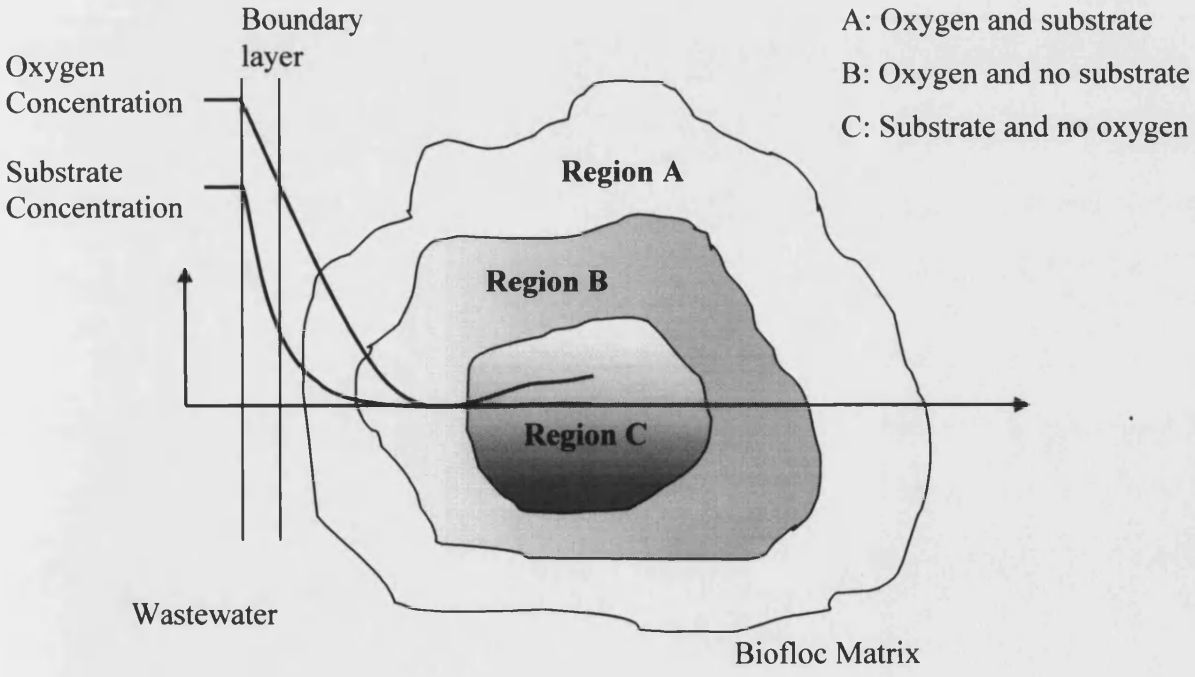


Figure 2.2: Oxygen and substrate concentration profiles within a biological floc matrix (Abbassi *et al.*, 1999)

The increased dissolved oxygen in the bulk liquid leads to a deep diffusion of oxygen into the biological floc, which subsequently results in an enlargement of the aerobic volume within the floc. This in turn leads to a further degradation of the hydrolysed bacterial cell in the floc matrix. However, oxygen becomes limiting in the centre of floc and substrate concentration starts to increase in the direction of the floc core as a result of the lysis process (i.e. destruction of the protoplasm area of the dead cells). By increasing the oxygen concentration, a further metabolism of the degradation of the protoplasm substrate from the dead cells occurs. Mathematical model results have shown that a rise in oxygen concentration causes a lower substrate concentration in the floc (Abbassi *et al.*, 1999). This leads to a lower growth rate and hereby a diminution of the excess sludge production. The soluble substrate released from the hydrolysis or oxidation of biomass is also known as the secondary substrate. The process whereby decay is followed by the growth on the secondary substrate is known as the death-regeneration/cryptic growth process. By promoting the cryptic growth phenomena, the total carbon source present in the influent would be reused over and over again, resulting in a reduced excess sludge production in terms of carbon balance.

However, Liu and Tay (2001) expressed their doubts in the results obtained by Abbassi *et al.* (1999). They highlighted the yield values obtained by Abbassi *et al.* (1999) being two-fold lower than the typical 0.6 mg MLSS/mg BOD_5, as reported by Boon and Burgess (1974) and McWhirter (1978) in pure oxygen processes. They commented "it is hard to imagine that over half of the sludge produced is decomposed through autohydrolysis of biomass at high DO concentrations, thus the mechanism proposed by Abbassi *et al.* (1999) needs further investigation".

2.3.2 Effects of Sludge Loading Rate (F/M Ratio)

The sludge retention time (SRT) or sludge age is a critical operational parameter in the wastewater treatment processes as it is also a control parameter that determines the microbial population growth in the process. In activated sludge theory, sludge retention time is defined as the average time a unit of biomass remains in the treatment system. An increase in the sludge age decreases the sludge loading rate, or better known as food to microorganism ratio (F/M), and leads to a reduced sludge production. This is due to the amplifying of cell endogenous death or lysis. Within the

literature, Stall and Sherrard (1976) reported that excess sludge production was reduced by 60% when the SRT was increased from 2 to 18 days, but no effect on COD removal efficiency was observed. In a high-purity oxygen activated sludge system, Wunderlich *et al.* (1985) reported a reduction of sludge production from 0.38 to 0.28 mg VSS/ mg COD removed as the SRT increased from 3.7 to 8.7 days. Based on these results, Liu and Tay (2001) pointed out that pure oxygen aeration process operated at a relatively long SRT would be beneficial for the reduction of excessive sludge production. SRT is controlled by varying the sludge wastage rate, given by D_w in the system.

The organic substrate removal rate and the sludge yield are greatly dependent on the viability of the cells. Viable biomass is defined by the ability of the biomass to consume substrate for its own active growth, which results in an increase in both the numbers and the specific weight of cell. Canales *et al.* (1994) investigated the effects of sludge age by varying D_w and found that biomass viability was greater with a higher wasting rate, D_w (or a smaller sludge age, SRT). In contrast, at a higher sludge age, the biomass was found to have a low viability level and COD removal associated with requirement for maintenance was higher than for growth, leading to a lower sludge production rate. The maintenance concept is discussed more thoroughly in a later section.

2.3.3 "Autodigestive" Process with Thermal Treatment of Sludge

Any means to intensify *in situ* cell endogenous respiration is essential in order to ensure a lower excess sludge production in a wastewater treatment process. According to Mason and Hamer (1987), the biodegradation of the cell wall is a rate-limiting step. Haug *et al.* (1978) and Hiroaka *et al.* (1985) discovered that the addition of a thermal pre-treatment improves the sludge digestibility. Hence, the possibility of an in-line sludge thermal treatment loop in the MBR is considered in this section.

An "autodigestive" process with the addition of a thermal treatment of sludge and the recycling of hydrolysates to the bioreactor was employed to study the temperature effects on the sludge production yield in an MBR (Canales *et al.*, 1994). It was observed that the addition of the thermal treatment loop improved the endogenous metabolism by cryptic growth with both low HRT (1 to 2 hours) and low sludge age

(10 hours). As a result of the sludge thermal treatment, a 3-fold increase of the maintenance coefficients and a 2.5-fold decrease of the biomass/substrate conversion yield were observed.

Efficient biomass death can be enhanced by thermal treatment at very fast rates. Heating the biomass for 10 minutes at 90 °C resulted in a biomass viability lower than 0.01% (Canales *et al.*, 1994). The analysis on the intracellular products released indicates an increase of TOC and polysaccharide release (due to cell lysis) but a decrease of protein release (which can be explained by denaturation), with a variation of temperature from 80-100 °C. These results show that sludge thermal treatment leads to a significant decrease of sludge production yield by biomass cryptic growth induction, with high metabolic activity and high purification yield.

2.3.4 Cell Breakage Techniques (thermal, alkaline, acid)

As discussed before, any approach that involves the reduction of the global substrate-to-biomass (F/M) conversion yield would result in a decreased sludge production. Rocher *et al.* (1999) stated that microbial cell lysis can be amplified to enhance cryptic growth (biomass growth on lysates). Cryptic growth consists in the ability of microorganisms to assimilate the intracellular products of cells. They studied different cell breakage techniques (thermal, alkaline, acid) on both the pure strain of *Alcaligenes eutrophus* and mixed population activated sludge in terms of generating lysates and evaluating their biodegradability. According to their work, Rocher *et al.* (1999) reported the dissolved organic carbon (DOC) releasing reaction to be a distinct two-phase process: first, a rapid step, during which the DOC release was a function of the treatment conditions, then a slower second phase (post-treatment release). They found the optimal conditions to induce cell breakage to be a pH of 10 at a temperature of 60°C for 20 minutes. The optimal degradation yield, obtained with activated sludge cells, reached 55% after 48 h of incubation and 80% after 350 h. Therefore, a combined thermal alkaline waste activated sludge pre-treatment can provide a viable alternative to other sludge pre-treatment processes and these cell lysis conditions can be transposed to increase the sludge solubilisation.

2.4 Process Design

2.4.1 Oxic-Settling-Anaerobic (OSA) Process

Oxic-settling-anaerobic (OSA) process is a modification of conventional activated sludge technology by inserting an anaerobic stage in the recycling bypass of sludge. Westgarth *et al.* (1964) reported that the insertion of a period of anaerobiosis in the high-rate activated sludge process could reduce the rate of excess sludge production by half as compared with a conventional process without anaerobic reactor. The concept of this was based on the approach of fasting/feasting and was reported to be an effective strategy in the activated sludge process (Chudoba *et al.*, 1992a; Ghiglizza *et al.*, 1996; Copp and Dold, 1998; Chen *et al.*, 2001).

For aerobic microorganisms, ATP is generated from the oxidation of exogenous organic susbstrate. When the microorganisms are subject to anaerobic starvation condition without food supply, they have to use their ATP reserves because they can no longer produce the energy. During this fasting period, the ATP would be exhausted. After returning to food-enriched aerobic reactor, they have to rebuild necessary energy reserves prior to biosynthesis because cellular synthesis could not proceed without a certain intracellular stock of ATP. In this case, the substrate consumption should thus go to catabolic metabolism to satisfy the energy requirement of microorganisms (Chudoba and Capdeville, 1991a). Therefore, it appears that alternative aerobic-anaerobic cycling of activated sludge would stimulate catabolic activity, and dissociate catabolism from anabolism. The energy uncoupling induced by this process design would lead to a minimized sludge yield.

Chudoba and Capdeville (1991a) and Chudoba *et al.* (1992a) found that in the case of oxic-settling-anaerobic process, the specific sludge production was reduced by 20-65% as compared to conventional activated sludge process. They also reported lower SVI value, which means an improved settleability of activated sludge. Ghiglizza *et al.* (1996) also reported successful application of oxic-settling-anaerobic strategy in sequencing batch reactor (SBR) system in terms of lower sludge production and excellent settleability.

The oxic-settling-anaerobic process proves to be a promising strategy in handling high-strength organic pollutants without serious sludge-associated problems, while improving the stability of process operation. However, the insertion of an anaerobic stage will add on to the capital cost, and may not be so easily implemented into the existing act ivated sludge wastewater treatment plant.

2.4.2 Ozonation

Sakai *et al.* (1997) reported their experience of using ozonation to reduce excess sludge production in a full-scale plant, treating 450 m^3/d municipal wastewater, which had been operated successfully for 9 months. They claimed that an activated sludge process that produces no excess sludge was possible. The working principle for the ozonation-combined AS process was proposed as follows, (i) a small amount of activated sludge taken from the aeration tank is ozonated in the ozone reactor. The ozonation enhances biodegradability of activated sludge and most microorganisms would be killed and oxidized to organic substances, (ii) the organic substances were then returned to the aeration tank to be further degraded and biologically oxidized. The amount of sludge elimination in this process is dependant on the ozone dosing rate and the amount of return sludge to be treated with ozone. Sakai *et al.* (1997) reported that a complete elimination of excess sludge is achievable when 4 times more amount of sludge than that of the excess sludge expected in the treatment without ozonation, is treated at an ozone dosing rate of 0.034 kg/ kg MLSS. Research by Kamiya and Hirotsuji (1998) showed that at an ozone dose of 0.01 kg/kg MLSS in aeration tank per day, the excess sludge production (ESP) was reduced by 50%, while complete elimination of excess sludge production was achieved with ozone dose of 0.02 kg/kg MLSS. Both sets of researchers reported that the effluent quality in terms of BOD and nitrogen was not influenced significantly. Moreover, according to Kamiya and Hirotsuji (1998), the sludge settleability in terms of SVI was highly improved as compared with control test without ozonation. Sakai *et al.* (1997) reported effluent suspended solids to be 2 – 15 mg/l higher compared to a control without ozonation, but this was still below the discharge limit. According to Liu and Tay (2001), ozonation could be a potential tool for reducing ESP, however further research is needed in order to find the optimised ozone dosage and dosing mode.

2.4.3 Chlorination

Saby *et al.* (2002) investigated the feasibility of using a chlorination step as an alternative solution to replace ozonation in the minimisation of excess sludge production. They claimed that although ozonation treatment is a potential and effective solution in reducing the excess sludge production, nonetheless the operational cost is significantly higher because both ozone generation and application are expensive. On the other hand, chlorine, like ozone, is also a strong oxidizer and chlorination operation cost is only 10% of that of ozonation in terms of disinfection practice (Metcalf and Eddy, 1991). In their research, Saby *et al.* (2002) operated two identical activated sludge membrane bioreactors continuously with synthetic wastewater under the same operating conditions for several months. The excess sludge was treated with chlorine in a separate reactor. After chlorination, the sludge return to the activated sludge system was conducted for 20h in order to lessen the impact of chlorine residuals in the liquor. At chlorine dose of 0.066 kg Cl_2/ kg MLSS, a 65% reduction of excess sludge was possible. The principal disadvantage of the chlorination treatment is the formation of trihalomethanes (THMs). However, Saby *et al.* (2002) reported less than 200 ppb THMs were detected in the effluent, possibly due to the volatisation of THMs during the chlorine treatment using a sprinkler system to eliminate sludge foaming problem. However, the chlorination treatment resulted in poor sludge settleability, as well as significant increase of soluble COD concentration in the effluent. Nevertheless, Saby *et al.* (2002) suggested and demonstrated that the integration of a membrane filtration module in the conventional activated sludge process could overcome these problems effectively.

2.5 Maintenance Concept

The maintenance concept, first proposed by Pirt (1975) is where microorganisms and cells utilize the energy generated from catabolism both for maintenance functions and for fueling the anabolism of new cells from the metabolites. The specific maintenance requirements known include the turnover of cell materials, osmotic work to maintain concentration gradients across the cell and also cell motility.

2.5.1 Effects of Sodium Chloride Addition to Maintenance Energy Requirements

Cells were found to satisfy their maintenance energy requirements prior to utilising energy in anabolism (Low and Chase, 1998). In order to reduce the excess sludge production, wastewater treatment process must be engineered in a way that pollutants are diverted from assimilation via biosynthesis, to non-growth associated energy consuming functions (Low and Chase, 1999a). One way to enhance the maintenance energy requirements of the cells is by addition of sodium chloride. Strachan *et al.* (1996) proposed that by the addition of sodium chloride, the maintenance energy requirement of the cells was affected through the increased energy needed to pump the sodium out of the cell. With the extracellular sodium ions (Na^+) concentration being much greater than the intracellular concentration, sodium ions diffuse readily into bacterial cells via a passive or facilitated transport. As Na^+ ions are toxic to the cells, energy must be expended for the active transport of Na^+ ions out of the cells against a concentration gradient. This additional usage of energy manifests itself as an increased maintenance energy requirement. This would consequently result in a decreased biomass yield, as less carbon source is available for anabolism in a substrate-limited situation.

In a culture of *Saccharomyces cerevisiae*, Watson (1970) observed that the addition of 1 M NaCl increased the maintenance energy requirements and led to a consequent decrease in the observed biomass yield. Strachan *et al.* (1996) also reported on a steady increase in maintenance energy requirement with the addition of NaCl up to a concentration of 30 g/l. Their results suggested that the addition of sodium chloride to the biomedium in a carbon-limited situation could indeed control the rate of growth of a biofilm. However, Hamoda and Al-attar (1995) found that the constant addition of NaCl (up to 30 g l^{-1}) did not have an effect on the reduction of biomass, though the organic removal efficiency and the effluent quality did not deteriorate. It was suggested that the biomass had adapted to the saline environment during acclimatisation.

It is also important to consider the effects of sodium ion concentrations, since an inadvertent increase may significantly affect the energetic of the cellular growth. It was found that at very high concentrations of sodium ions, the bacterial cells become

inactivated when they can no longer pump out sufficient sodium ions against the concentration gradient.

Further research needs to be carried out to investigate the feasibility of this technique applied to mixed microbial populations. Also, different species of bacteria will react differently and have a different tolerance limit to the maximum concentrations of sodium chloride. Hence, a salt concentration that reduces the growth rate of one strain may be sufficient to result in the death of another strain, thus altering the balance of populations in the mixed culture. Therefore, care should be taken into consideration when designing the technique applied to a specific wastewater and microbial community. However with sufficient investigations on the applicability of sodium chloride addition, this technique may prove to be a cheap, non-toxic and effective method for controlling the excess sludge production in wastewater treatment processes, thus greatly alleviating and enhancing the performance of the process plant.

2.6 Mechanism of Uncoupled Oxidative Phosphorylation

2.6.1 Microbial Energy Generation

The heterogenous microbial population in the wastewater treatment process can utilise a wide range of substrates. The sole purpose of substrate utilization is to generate the energy needed for growth, reproduction and maintenance functions. A series of microbial metabolisms converts the chemical and physical energy generated into biologically utilisable energy and this energy is stored as the energy-carrying compound known as adenosine 5'-triphosphate (ATP). It has a molecular structure consisting of an adenosine molecule linked to three inorganic phosphate molecules by phosphoryl bonds. The formation of the bond requires a large input of energy. Hence, similarly the hydrolysis of this bond will liberate energy useful for utilization by the microorganism, which is termed as "free energy". This free energy fuels endergonic functions such as cell anabolism, reproduction, cell motility and other maintenance functions such as active transport of substrate and regulation of intracellular concentrations (Low and Chase, 1999b).

Chemoorganotrophs are capable of oxidising a large array of carbon substrates as carbon and energy sources (Horan, 1990). The ultimate aim for energy yielding

catabolic pathways is to provide energy for the synthesis of ATP. Oxidation proceeds in a series of reactions catalysed by enzymes, which are organised to release the energy in discrete and controllable amounts enabling them to be coupled to ATP synthesis. Three alternate metabolic pathways have been identified in chemoorganotrophs for reducing organic compounds. These pathways differ in their initial reactions but share a common mechanism for the conversion of the phosphorylated three-carbon sugar, glyceraldehyde 3-phosphate, to pyruvate. One of the most widely used mechanisms is known as the glycolysis or the Embden-Meyerhof-Parnas (EMP) pathway. The bacterial genus *Pseudomonas*, a significant oxidiser of carbon in wastewater treatment process, utilise the Entner-Doudoroff pathway which is similar to glycolysis, but is less efficient in ATP generation.

The major reactions of catabolism are oxidation reactions and these involve the removal of hydrogen or electrons. Two most common acceptors for these reducing equivalents in biological systems are nicotinamide adenine dinucleotide (NAD^+) and its phosphorylated product $NADP^+$. As catabolic activity is in excess of anabolic activity in growing cells, an excess of reducing equivalents is produced. As NAD^+ and $NADP^+$ are present only in catalytic amounts, the respiration process is utilised to reoxidise NAD(P)H back to NAD(P). The reducing equivalents are passed from NAD(P)H through a series of electron carriers to an ultimate electron acceptor. This series of carriers are redox couples of increasing redox potential, collectively known as the electron transport chain. The exact mechanism of coupling was proposed by Mitchell (1972) and is known as the chemiosmotic process of oxidative phosphorylation. This theory proposes that the inner membrane of the eukaryotic mitochondria and the prokaryotic cell is impermeable to the passage of ions including H^+ and OH^-. As a result of the spatial orientation of the protein carrier molecules in the chain, the transfer of electrons will generate a gradient of pH and electrical potential across the cell membrane. The enzyme complex ATPase is capable of coupling the movement of H^+ across the membrane to ATP synthesis, and this enzyme is also the means whereby the energy, which is stored as an electrochemical gradient, is released. The proton-motive force is essential in providing the activation energy in the phosphorylation of ADP to create a high free energy covalent bond in ATP.

Intracellular regulation of catabolic and anabolic activities by bacteria is necessary to ensure an efficient flow of energy. It was discovered that within the mitochondria of higher organisms, the concentration of ATP is known to inhibit activity in the energy generating tricarboxylic acid cycle (TCA), in effect producing a feedback control loop (Stryer, 1988). However, the presence of respiratory control in bacteria is uncertain. Senez (Senez, 1962) suggested that bacterial anabolism is coupled to catabolism of substrate through rate limiting respiration. Nevertheless, an uncoupled mechanism would also occur if respiratory control did not exist and instead the anabolic activity is rate limiting. Hence, excess free energy would be diverted from the production of biomass. To consume this available energy, several possibilities were considered, including the dissipation of energy as heat by adenosine triphosphatase systems, the activation of alternative metabolic pathways bypassing free energy conserving reactions and the accumulation of polymerised products in storage forms or as secreted waste (Low and Chase, 1999b).

Stouthamer (1979) reported that uncoupled mechanism had been observed:

1. in the presence of inhibitory compounds
2. in the presence of excess energy source
3. at unfavourable temperatures
4. in minimal media during transition periods, where cells are adjusting to changes in their environment

The following section will look at the definition of uncoupled mechanism in more detail.

2.6.2 Definition of Uncoupled Oxidative Phosphorylation

Russel and Cook (1995) defined "uncoupling" as being the inability of chemiosmotic oxidative phosphorylation to generate the maximum theoretical amount of metabolic energy in the form of ATP. The ATP lost to non-growth reactions is termed as ATP spilling. Decreasing the ATP available for biosynthesis will reduce the production of excess biomass and ability to replicate these uncoupling processes in wastewater would therefore be advantageous. Furthermore, if microorganisms exhibit similar behaviours to mitochondria in the regulation of the activity in the TCA cycle, then a

reduction of cellular ATP concentration would provide a stimulus to the feedback control loop to promote catabolism of the pollutants.

2.6.3 Inducing Uncoupled Oxidative Phosphorylation

Uncoupling of the oxidative phosphorylation can be induced by dissipating the proton motive driving force. This can be done by increasing the proton-conducting capacity of the membrane. Zakharov and Kuz'mina (1992) suggested that elevated temperatures increase the proton permeability of the membrane from their observation on *Thermus thermophilus*.

2.6.3.1 Addition of Organic Protonphores

Research on the uncoupler-induced energy spilling process had been carried out and reported in the literature (Okey and Stensel, 1993; Low and Chase, 1998; Chen *et al.*, 1999; Mayhew, 1999; Strand *et al.*, 1999; Liu, 2000; Low *et al.*, 2000). The use of organic protonphores or uncouplers such as 2,4-dinitrophenol (*d*NP), para-nitrophenol (*p*NP), pentachlorophenol, 2,4,5-Trichlrophenol (TCP), 4-chloro-2-nitrophenol (CNP) and 3,3',4',5-tetrachlorosalicylanilide (TCS) had been investigated and reported by different researchers.

Low and Chase (1998) investigated the use of protonphoric uncouplers, 2,4-dinitrophenol (*d*NP) in a chemostat pure culture of *Pseudomonas putida* and reported it to be less effective compared to para-nitrophenol (*p*NP). Using 30 mg/l para-nitrophenol, a sludge yield of 0.15 was obtained compared to sludge yields of 0.32 to 0.35 for the control samples. Low and Chase (1998) found that with the supplement of 100 mg/l para-nitrophenol in the feed, the specific substrate uptake rate was increased and the efficiency of biomass production was reduced by 62%. This is due to the effect of energy (ATP) dissipation within the cells and thus reducing the energy for endothermic processes. Oxidation of the substrate still occurs but the phosphorylation of ADP to ATP is reduced (Simon, 1953). Thus, the cells satisfy their maintenance energy requirements prior to utilising energy in anabolism. Low and Chase (1998) also found that a lower pH of 6.2 enhanced the uncoupling effect, resulting in a sludge reduction of 77%. Simon (1953) also reported that acidic conditions improved the uncoupling activity of organic protonphores.

Strand *et al.* (1999) studied the effects of various uncoupling chemicals on observed yield of an activated sludge batch culture degrading glucose and reported that 2,4,5-Trichlorophenol (TCP) and 4-chloro-2-nitrophenol (CNP) to be the most effective uncouplers in short-term tests. TCP also functioned as an effective uncoupler during long-term operation of a continuously fed completely mixed activated sludge system when 10 mg/l was added to the influent. The MLVSS decrease was steady at 60%. The minimum concentration required for uncoupling was > 2.0 mg/l, below which after 80 days microbial populations had developed to be resistant to the uncoupling effect of TCP and capable of more efficient TCP degradation.

Chen *et al.* (2002) studied the feasibility of using 3,3',4',5-tetrachlorosalicylanilide (TCS) as a metabolic uncoupler to reduce sludge growth in batch activated sludge cultures. They found that the TCS threshold concentration of triggering a reduction in sludge growth to be 0.4 mg/l. When the TCS concentration was 0.8 – 1.0 mg/l, the reduction in sludge growth was reported to be around 40%. The 1.0 mg/l TCS concentration increased the microbial activity by 42% and increased the active cell percentage by 3 – 4%. The effectiveness of TCS can be sustained during the 30-day operation of activated sludge batch culture without detriment to the treatment performance. The use of TCS stimulates energy spilling or dissipation by reducing the cell membrane resistance to the proton transfer between the bulk solution and cells. This led to inefficient ATP synthesis, which subsequently resulted in a reduced cell synthesis, as cells would satisfy the maintenance requirement prior to anabolism. The use of TCS was said to be more environmentally sound among other uncouplers as it has been often used as a component in formulation of soaps, rinses, shampoos, etc. (Budavari *et al.*, 1989; Liu and Tay, 2001).

The addition of protonphores might prove to be an effective and direct mechanism of minimising the excess sludge production without reducing the rate of organics removal from the wastewater. However, the use of organic protonphores still requires review, as protonphores would need to be removed from the treated water prior to discharge due to their inherent toxicity. Final polishing, such as ozonation, may be required to 'clean' the effluent prior to discharge. Mayhew (1999) suggested that chemical uncoupling might be the simplest strategy of biomass reduction in plants where processes like ozonation are already in use, as it requires no additional plant or

process changes. Other issues with uncoupler addition are that cultures might become acclimatised to the uncoupler in long term operations, in which a microbial population that was able to degrade the uncouplers more effectively develops (Strand *et al.*, 1999). Strand *et al.* (1999) suggested adding the uncouplers intermittently or to alternate between various uncoupling chemicals. This, however, may lead to unacceptable levels of uncoupling chemicals in the effluent. Further research is necessary to determine the long-term application of chemical uncouplers to minimise sludge production in wastewater treatment plants.

2.7 Processes and Mechanisms involved in Sludge Reduction

From the practical operating experience of wastewater treatment plants, it is clearly understood that an increase in sludge retention time or sludge age (SRT) is accompanied by a decreased sludge production. This decreased biomass yield is typically attributed to mechanisms such as maintenance energy requirements, decay or lysis of cells due to adverse environmental conditions (pH, toxic substances or temperature), grazing by protozoa and metazoa, endogenous respiration, death-regeneration or decay-cryptic growth (Van Loosdrecht and Henze, 1999). These are the different sets of physiology and metabolism occurring in the second stage of the system and they are explained in the following sections.

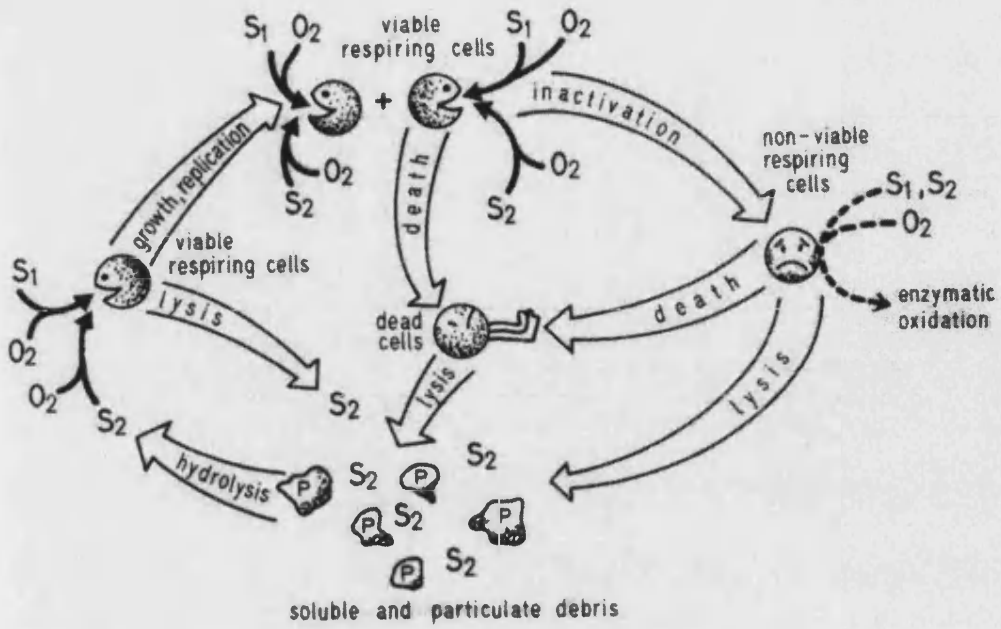
2.7.1 Endogenous respiration

Endogenous metabolism or respiration of microorganisms may be defined as "the total metabolic reactions occurring within the living cell in the absence of compounds or elements which may serve as specific exogenous substrates" (Dawes and Ribbons, 1962). Over the past century, many papers and data have been published on the findings and elucidation of the concept of microbial death. However, it has proved a difficult task with many differing opinions. Especially in the wastewater treatment and sludge digestion processes, the term endogenous metabolism has been used rather more loosely whereby it was used to embrace several additional phenomena that are clearly outside the traditional definition, in which it was explained on the basis of the maintenance functions of microbes, i.e. turn-over of cell materials, osmotic work to maintain concentration gradients between the cell and its exterior and cell motility (Hamer, 1985). These include phenomena such as the utilization of adsorbed/absorbed substrates or entrapped particulate substrates associated with activated sludge flocs,

the utilization of intra-cellular storage products that has formed in microbial cells under specific nutrient limitations, uncoupling of growth and respiration due to nutrient pulses and the occurrence of microbial death, lysis and "cryptic" growth.

In the natural environment, it is unavoidable for microbes to undergo adverse conditions such as starvation, lack of nutrients, physical environment changes (T, pH, etc.). A culture of microbes is composed of various morphological, biochemical and physiological groups (Mason *et al.*, 1986a). Microorganisms have been classified on a physiological basis as: (a) dead microbes; (b) non-viable, active or respiring microbes; (c) dormant microbes; (d) viable, active or respiring microbes. Understanding these definitions will assist us in the process of comprehending the different microbiological physiologies that are put forward in the later section.

The strict definition for dead microbes is that they are organisms totally devoid of metabolic activity, but still possessing a cell wall (Mason *et al.*, 1986a). Non-viable microbes are organisms that have lost the ability to reproduce due to genetic defects such as absence of a critical enzyme required for replication or lethal breaks in the DNA of the microbe, but still able to carry out substrate transformations. In other words, these are microbes still respiring but not producing new cells. Dormant microbes can be subdivided into two categories: spores and temporarily inactive or resting microbes. They are usually the outcome of an adverse environment such as the lack of food, etc. Spores serve distribution and survival functions, whilst resting microbes are only intermediates leading either to active microbes or to death or lysis (Koch, 1971). Active microbes are cells that are actively assimilating substrate and replicating, thus leading to an increase in cell mass. Figure 2.3 illustrates the different state of microbes and fundamental mechanisms involved with each state.



S_1 – Readily biodegradable substrate; S2 – Slowly biodegradable substrate and P – inert particulates / biomass

Figure 2.3: Physiologically differentiated forms of biomass present in a continuous culture system and the fundamental processes occurring (Mason *et al.*, 1986b).

2.7.2 Starvation

Starvation is a natural occurring phenomenon in the environment for the cells as they are often subjected to variations in substrate availability, (i.e., nitrogen, phosphorus), in T, in oxygen, and toxic chemicals as well as to spatial variations. If the cells are attached, then differences in substratum composition may also exist. Despite these conditions, microbes readily survive in their natural environments. Therefore, it is apparent that mechanisms exist conferring the necessary properties to compete and survive (Mason *et al.*, 1986a).

A microbial cell requires a carbon source, an energy source and nutrients in order to cater for biomass synthesis (anabolism) and metabolic regulation (catabolism). However, it is known that bacteria are able to survive, sometimes for very long periods, in the absence of any or all of these requirements. Suspensions of aerobic or facultative organisms in water, saline, or buffer solutions consume oxygen and

liberate carbon dioxide, which indicates that reserve materials within the cell are being oxidized, presumably to provide the energy necessary for survival (Dawes and Ribbons, 1962). For a cell to survive during starvation, only a very small part of its metabolic potential needs to be expressed. These have been collectively referred to as maintenance functions, and include maintenance of osmotic potential, turnover of essential cell materials, and maintenance of the membrane potential. If energy for these processes is not provided, it is said that the cell will irreversibly cease to function (Mason *et al.*, 1986a). The actual causes by starvation may be quite diverse and include factors such as the loss of the capacity for nucleic acid and protein synthesis. The loss of a single enzyme from the cell could lead to its "death" in certain media; this is clearly seen with techniques that select nutritional mutants via minimal media.

Maintenance energy in the absence of an exogenous energy source has to be derived from the oxidation of either endogenous cellular constituents or storage products. This degradation is known as endogenous metabolism and can be defined as the summation of all metabolic reactions which occur when a cell is deprived of either compounds or elements which may serve specifically as exogenous substrate (Dawes, 1976).

Wilkinson (1959) described three main classes of compounds that could easily act as energy-storage compounds. These are polysaccharides, lipids (including PHB), and polyphosphate; all occur in widely varying amounts dependent upon the particular species and the environmental conditions. However, it is apparent that there are many other substrates for endogenous metabolism; these include ribonucleic acid (RNA) and protein (both are subject to turnover) and free amino acid and peptide pools (Dawes and Ribbons, 1964). Possible substrates not yet implicated in endogenous metabolism include deoxyribonucleic acid (DNA), cell wall polymers and cell membrane materials. In the excess of external substrates (e.g. glucose), bacteria are capable of synthesizing the energy storage compounds.

Some microorganisms are endowed with the specialised mechanism of spore formation, which permits their survival under extremely adverse conditions: the available evidence suggests that nutrient deficiency, with consequent depletion of

cellular reserves, may initiate the process. Resynthesis or regrowth (also known as cryptic growth) at the expense of the dead and lysed products of other bacteria is also a possible phenomenon, which will be further looked into and discussed later.

2.7.3 Cryptic growth

Ryan (1959) first introduced the term "cryptic" growth and described the re-utilization of lysis and leakage products by intact cells of the same population as carbon energy substrates and nutrient sources. Mason and Hamer (1987) has highlighted the relevance and importance of the concept in mixed culture systems such as in wastewater treatment processes and other processes where biomass concentration subjects the organisms to extended periods of starvation within a recycle loop. Mason and Hamer (1987) argued that the discrepancy between the fundamentally derived biomass yield coefficients, i.e., the amount of biomass expected to be produced by calculation (Stouthamer, 1979) and the observed values for biomass yield have been explained in various ways under the term endogenous metabolism in which the maintenance energy contribution is just one factor. The processes of death and lysis and potential "cryptic" growth have been largely ignored despite the fact that such processes obviously can play a role in yield coefficient reduction. In continuous cultures, lysis has been suggested as being the major reason for the theoretical maximum biomass yield coefficient reduction, especially at low growth rates (Mason *et al.*, 1986b).

Mason and Hamer (1987) demonstrated the ability of *Klebsiella pneumoniae* to grow on its own soluble lysis products by carrying out a series of batch growth experiments. Growth of *K. pneumoniae* on sonicated cells was observed to grow with no lag phase, suggesting that "cryptic" growth is probably a real event occurring in growing chemostat cultures under ideal growth conditions and most probably also under starvation conditions. Different research studies had been carried out based on the concept of cryptic growth (growth on intracellular products) and these were very important findings that contribute towards the overall sludge reduction strategies (Canales *et al.*, 1994; Egemen *et al.*, 1999; Rocher *et al.*, 1999). The available sludge reduction strategies have been covered in an earlier section of this chapter.

2.7.4 Maintenance and Predation

In earlier sections of this chapter, the phenomena of maintenance and predation by higher trophic organisms have been covered.

2.7.5 Summary of Definitions

Due to a large variation in definitions used in the context endogenous respiration processes, Van Loosdrecht and Henze (1999) in their review paper have defined the different processes and mechanisms involved during the reduction of biomass mass in Table 2.1 and 2.2.

Table 2.1: Primary mechanism involved in reduction of biomass mass (Van Loosdrecht and Henze, 1999).

Mechanism	Description	Caused by
Maintenance	Energy consumption for cell maintenance under the use of external substrate or internal stored substrate such as glycogen or PHA	Basic metabolic energy requirements such as membrane potential, renewal of proteins, motility, etc.
Decay	Processes which reduce the weight and specific activity of biomass	Internal and external factors
Internal decay	Decay caused by cell internal activity	Death and self-oxidation of cell constituents
External decay	Decay caused by cell external factors	Predation
Lysis	Solubilisation of biomass, releasing secondary substrates into the liquid	Enzymes, pH, toxicants, viruses
Predation/ Grazing	Higher animals consuming microorganisms	Protozoa, metazoa, etc.

Table 2.2: Processes involved in the reduction of biomass mass (Van Loosdrecht and Henze, 1999).

Process	Description
Maintenance	Direct consumption of cell external or internal substrates for maintenance of the cell integrity
Endogenous respiration	Respiration with oxygen or nitrate using cell internal components
Death-regeneration, including lysis and growth (cryptic growth)	Cell lysis followed by growth on the secondary substrate arising from the decay

2.8 Conclusions

With the expansion of population and industry, an increase in excess sludge production is generating a real challenge in the field of environmental engineering technology. There is no single global solution for the problem with increasing sludge production, though Liu and Tay (2001) commented in their review paper that the activated sludge process combining membrane separation and physicochemical lysis has great potential in reducing sludge production. The different sludge reduction strategies reviewed are:

(i) ecosystem manipulation by protozoal grazing,

(ii) optimisation of process parameters (such as dissolved oxygen, temperature, F/M ratio, etc.),

(iii) process design (OSA, ozonation and chlorination),

(iv) maintenance energy and cryptic growth concept, and

(v) mechanism of uncoupled oxidative phosphorylation (addition of organic protonphores).

The different mechanisms involved in the reduction of sludge production as well as occurring in the second stage of MBR were also being outlined to increase the understanding from the microbiological point of view.

Chapter 3: Materials and Methods

Scope of Chapter 3

Two experimental areas were identified and carried out to achieve the aims of the project. These were a) to compare and evaluate the performances of the two stages as a whole as well as if they were to be individual units and b) to identify the different mechanism and biokinetics in the two systems. The set-up of the rig and the analytical equipment used to help towards the accomplishment of the project are outlined here.

3.1 Experimental set up of Membrane Bioreactor (MBR)

3.1.1 Design of the MBR rig

The laboratory-scale MBR rig was designed by the MBR team (Prof J A Howell, Dr T C Arnot, Miaw Ching Sim and Hwee Chuan Chua) and built at the University of Bath. It is a scale-down of a large-scale commercial MBR unit. The materials of construction used for the membrane bioreactor design are perspex sheets of 8-mm thickness. The transparent design of the reactor allows a clear observation of the system itself and also the air bubble pattern. The membrane bioreactor is a box with the following dimensions: H = 704 mm, L = 222 mm, W = 32 mm. The total working volume of the bioreactor is 3.5 litres, with the membrane and downcomer in place.

3.1.2 Process configuration of the MBR rig

Figure 3.1 shows the engineering drawing of the MBR rig design. The process configuration of the bioreactor is such that the flat-sheet membrane sits on the upper part of the reactor. The membrane is slotted into the reactor from the top, with groves on both sides of the inner plates of the reactor, each of 2 mm deep to hold the membrane in place. Another sheet of perspex, with the thickness of 6 mm and height of 540 mm, is placed next to the membrane with a spacing of 5 mm from the membrane and 10 mm from the side wall of the reactor. This will serve as a downcomer, in order to promote the liquid circulation inside the reactor (see Figure 3.2). The top plate of the membrane bioreactor has several ports where the temperature sensor, dissolved oxygen electrode, pH electrode are situated. The feed inlet, permeate outlet, vent gas outlet, sludge wastage outlet also have ports at the top plate of the bioreactors. The O-Ring around the box prevents leakage as the liquid

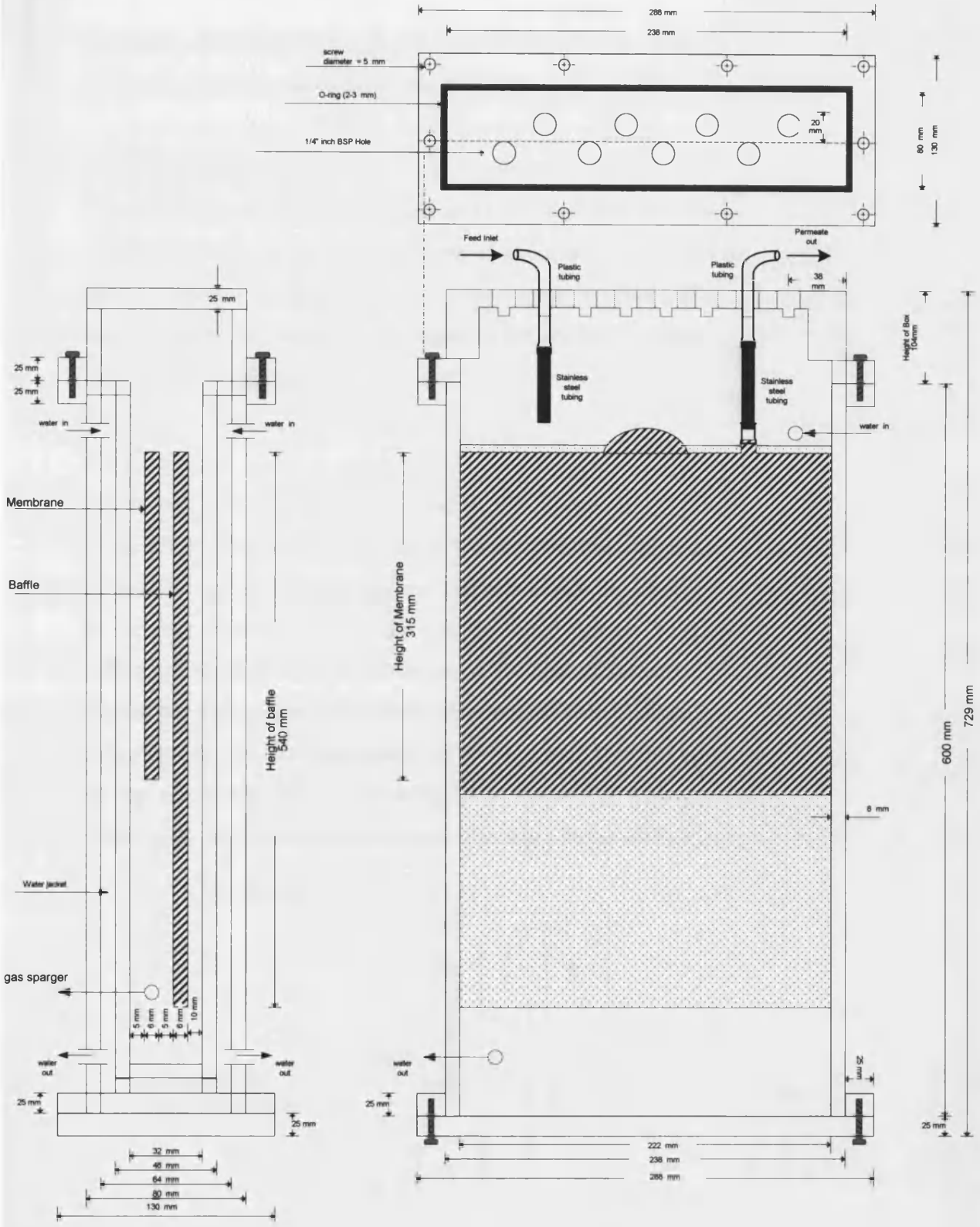


Figure 3.1: Engineering Drawing of the Lab-Scaled Membrane Bioreactor Design.

level in the bioreactor rises up to the top box. The top box is secured by bolts and nuts on the 25-mm thick flanges onto the bioreactor.

3.1.3 Temperature control

The membrane bioreactor is placed within another box with the same dimensions of the bioreactor itself, but with the width of 8 mm on both sides. This serves as a water jacket to control the temperature in the bioreactor. A Grant heater and water bath were used to pump hot water into the water jacket and recirculated to maintain the liquid temperature within the bioreactor at 20 ± 2 °C.

3.1.4 Aeration

The bioreactor is also designed based on the concept of airlift system. At the bottom of the reactor is an air sparger, which is a perspex tube with a diameter of 12 mm. The air bubbles rise up through the two channels beside the membrane sheet and the wall of the reactor and disengage through the liquid surface on the top. As the bubbles disengage, the liquid density at the top of the reactor increases. Hence, the liquid will flow down through the downcomer ensuring good circulation of the liquid (see Figure 3.2). Another box with dimensions of H = 104 mm, L = 238 mm and W = 80 mm sits on top of the reactor. This is designed to allow sufficient gas disengagement. The width of the box extended both ways, allowing a larger surface area.

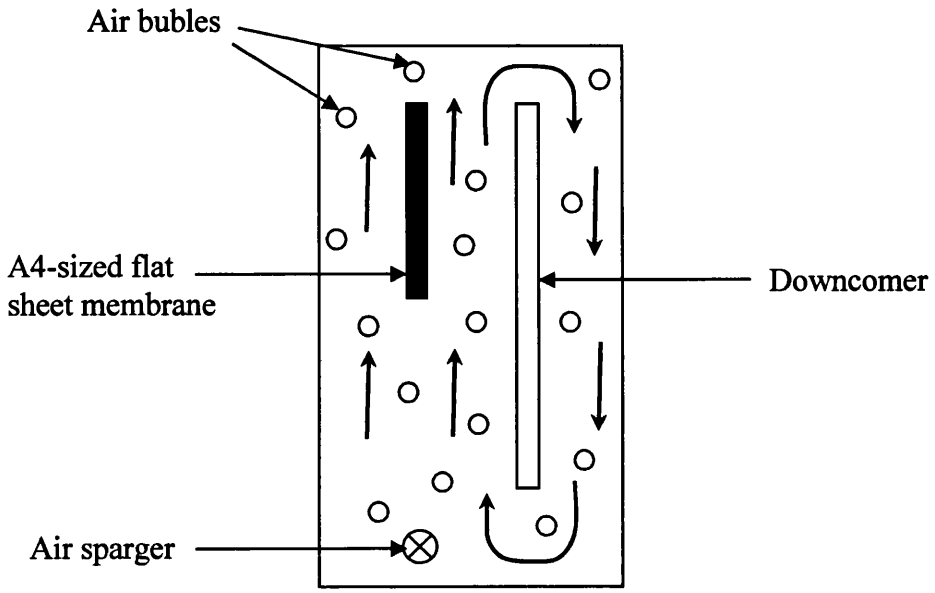


Figure 3.2: Pattern of recirculation of liquid and air bubbles in the MBR

The sparger has 4 air bubble holes, each with a diameter of 4 mm. This is to ensure the generation of coarse bubbles. Air bubbles serve in two functions in the MBR, i.e. to provide the oxygen required for the metabolism of the aerobic microorganisms and to generate an upward crossflow over the membrane surfaces, keeping fouling of the filtration surface to a minimum (Ueda *et al.*, 1996; Churchouse, 1997). This uplifting two-phase flow of bubbling air and mixed liquor provides a continual upward shearing flow that removes the cake layer deposited on the membrane (Ueda and Hata, 1999; Kolega *et al.*, 1991). However, the transport of oxygen to the intracellular sides of the microorganisms is diffusion-limited. Thus, the use of smaller air bubbles with a larger contacting surface area would improve the oxygen mass transfer efficiency (Horan, 1990). Nevertheless, the use of small air bubbles does not generate the slug upflow of liquid necessary to clean the membrane surface effectively. Therefore, a compromise will have to be made between the two purposes.

3.1.5 Membrane

A laboratory-scale, A4-sized Kubota flat sheet membrane, is immersed in the riser section. The membrane is made of polyolefin with a non-woven cloth base, giving a nominal pore size of 0.4 μm (Davies *et al.*, 1998). The membrane cartridge consists of solid acrylonitrile butadiene stryene (ABS) support plate with a spacer layer between it and an ultrasonically welded flat sheet membrane on both sides (effective filtration area = 0.106 m^2). Permeate passes through to the interior of the membrane and is pumped out via an outlet nipple cast on the top of the support plate by suction. Figure 3.3 shows a picture of the Kubota flat sheet membrane used in this project.

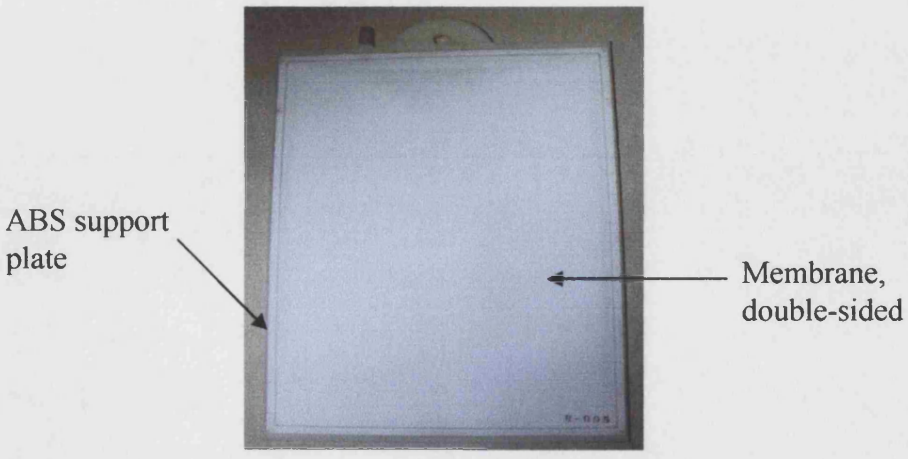


Figure 3.3: Lab-scaled, A4-sized Kubota flat sheet membrane

3.2 Synthetic sewage feed analogue

3.2.1 Medium composition

The proposed synthetic sewage feed analogue is derived from a recipe included in an OECD Technical Report (1976) on surfactant. This is the most widely used synthetic sewage recipe found from the literature, representing settled sewage after primary settling. However, the results of analysis with the suggested recipe were reported by Cranfield University to be insufficient to meet the target COD, as being discussed at the 2nd MBR-T meeting (2000) held at Imperial College, London. In order to provide a comparative analysis of the results obtained with other liased researchers from Imperial College and Cranfield University, the standardised recipe was revised as follows, whereby the COD strength is the medium value of untreated domestic wastewater (Metcalf and Eddy, 1991):

- COD = 500 $mg.l^{-1}$
- COD:BOD:TN:Ammonia:P = 140:65:11:7:1.5-2

The materials (peptone, meat extract) and AnalR grade chemicals are supplied by Merck Ltd, Poole.

Table 3.1: Original and modified OECD recipe for the synthetic sewage feed analogue.

	Objectives	Original recipe	Revised recipe
Peptone	-	1.6	2.0
Meat Extract	-	1.1	1.4
Urea	-	0.3	0.1
$CaCl_2.2H_2O$	-	0.04	0.04
$MgSO_4.7H_2O$	-	0.02	0.02
K_2HPO_4	-	0.26	0.11
NaCl	-	0.07	0.07
COD ($mg.l^{-1}$)	**500**	346±8	460±34
TN ($mg.l^{-1}$)	**39**	58±3	44
P ($mg.l^{-1}$)	**7**	16.3±0.5	10.5

Note: Concentrations of the recipe components are given in $g.l^{-1}$. COD, BOD, TN, P are given in $mg.l^{-1}$. Standard deviations are given where tests were conducted in duplicate.

3.2.2 Preparation and sterilisation of medium

Feed medium was prepared with deionised water in a ten times concentrated stock in a 10-litre aspirator tank. The feed medium was then autoclaved at 121°C and 1 bar for fifteen minutes. The possibility of protein degradation in the medium due to high temperature sterilisation had been discussed. However, the feed stock was very concentrated and organically rich that it was necessary to sterilise the medium. Hence, the above methodology of sterilisation was agreed among the researchers from Cranfield University and Imperial College in the project team.

3.2.3 Delivery of medium

The concentrated feed stock was delivered with a syringe pump at a very small flowrate of 1 ml/min. It was diluted down to a ratio of one to nine with a T-connector connecting both the deionised water line pumped with a peristaltic pump and the concentrated feed stream. The mixture was then delivered into the 1st stage (MBR 1).

3.3 Control system of the MBR

3.3.1 Interfacing and software

A physical interface between the membrane bioreactors and a desktop computer was established for data logging and analysis. All of the measuring electrodes were connected to an interface box and then to the Viglen Pentium I processor via the GENIEDAQ interface card. The interface card had two signal output channels and 16 signal input channels, which allowed communication to be made between the PC and the individual units. The corresponding GENIEDAQ software allowed programs to be written up for data acquisition and control. Figure 3.4 showed the schematic diagram for the membrane bioreactors.

3.3.2 Measurement of parameters

The FISHER pH electrode, FARNELL temperature probe and the UNIPROBE dissolved oxygen electrode were used to read the pH, temperature and dissolved oxygen of the system respectively. The in-situ pH electrode and dissolved oxygen electrode were fouled easily in the membrane bioreactors as biomass clogged the electrodes. Therefore, the probes needed to be calibrated from time to time. pH was also measured ex-situ when the sludge sample was withdrawn for the determination of the biomass concentration.

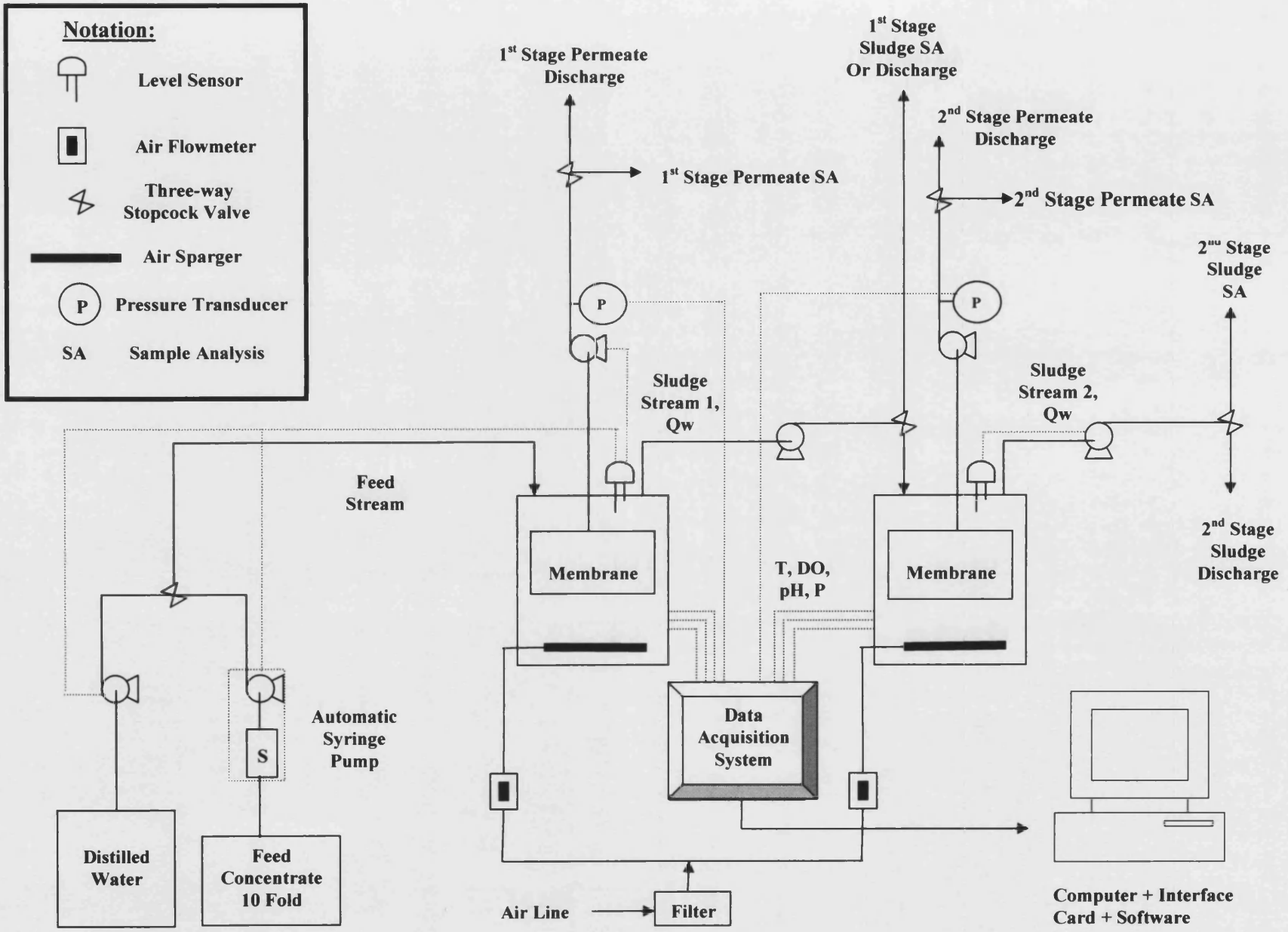


Figure 3.4: Schematic Diagram of the Control System for the Two-Stage MBR System

3.3.3 Level Control

The system experienced a few problems with the pumps and it was necessary to have a proper level control to maintain the MBR at a constant volume. Two control systems were employed. The finer control whereby a pressure transducer was used to measure and control the pressure head of the liquid in the bioreactor. If the level of the bioreactor dropped, the pressure transducer sensed the change and signal was sent to adjust the speed of the water feed pump. This way, the system will always be maintained at a constant volume. The emergency control was made up of three stainless steel tubes, which serve as conducting media. One was the common point, one was the H-H (High-High) point and the other was the H-L (High-Low) point. If the level rose higher than the designated H-H point, the circuit would be closed. This would cut off the power supply to both the feed syringe pump and water pump. Likewise, if the level dropped below the H-L point, the circuit would be disconnected and the permeate pump will stop pumping. The pumps would resume operation when the level reached its set point.

3.4 Operating Conditions of the MBRs

3.4.1 Pumping

Watson-Marlow 101U peristaltic pumps and 4-mm silicone tubing were used for the delivery of feed to the MBR system. Water was pumped from a large water reservoir and joined the concentrated feed stock at the T- connector into the first stage of the MBRs. Permeate was withdrawn by suction with the peristaltic pump at a flux of 5.5 LMH, corresponding to a HRT of 6 hours. Sludge was wasted from the first stage into the second stage with an automatic timer where 36 ml of sludge was pumped out every four hours, corresponding to a SRT of 16 days.

3.4.2 Gas flowrate

Air was pumped into the membrane bioreactors at a flowrate of 10 LPM (see Chapter 1). There was a filter unit along the air line in the laboratory to prevent dirt and grease in the air. The flowrate was read by a Cole-Parmer air flowmeter (range: 0 – 10LPM) and the dissolved oxygen concentration in the bioreactors was maintained above 2 mg/l.

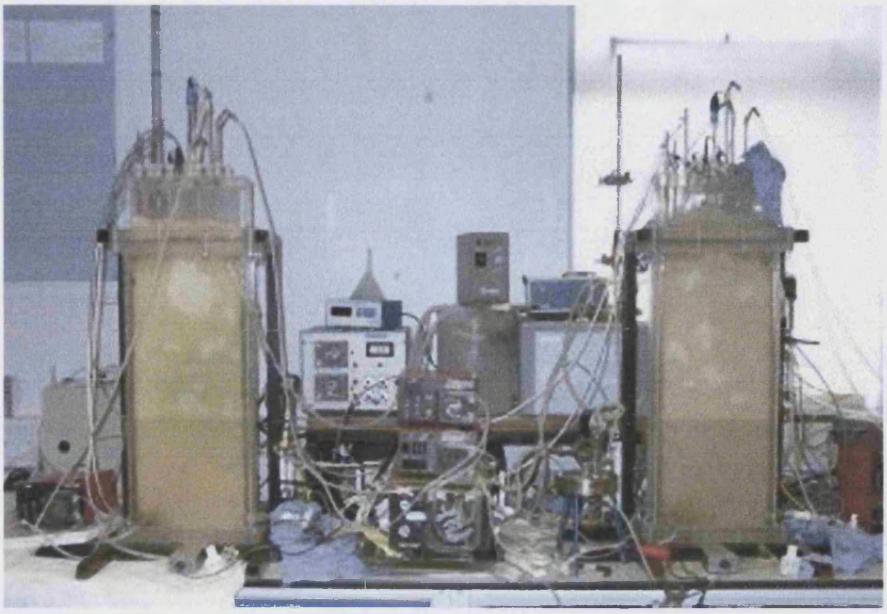
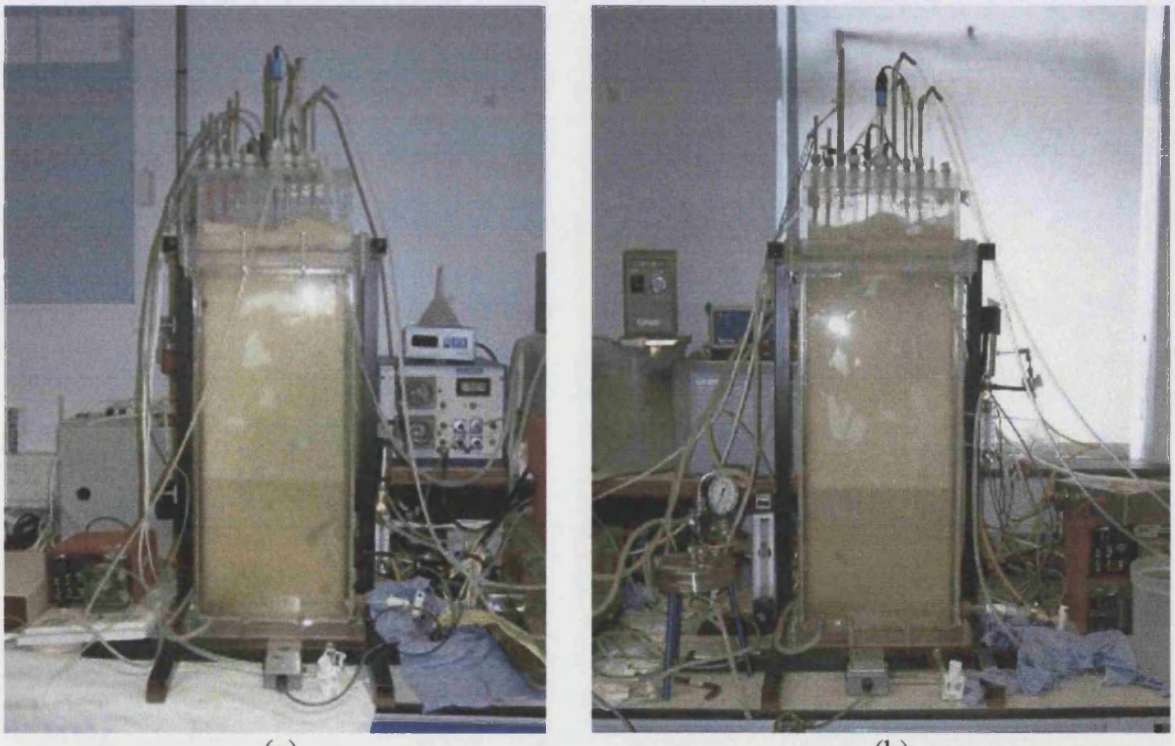


Figure 3.5: Two-stage membrane bioreactors (MBR) in operation



(a) (b)

Figure 3.6: (a) 1st stage MBR. (b) 2nd stage MBR

3.5 Microbiology of MBRs

3.5.1 Inoculum

The membrane bioreactors were inoculated with activated sludge sample obtained from the secondary activated sludge tank of the sewage treatment plants operated by Wessex Water in Cam Valley Sewage Treatment Work. The first stage of the bioreactors was started up with 1 litre of the activated sludge with an approximate mixed liquor suspended solids (MLSS) concentration of 3.5 g/l mixed with 2.5 litres of feed medium. The bioreactor was then allowed to acclimatise to the synthetic feed medium. The second stage of the bioreactors started receiving sludge wasted from the first stage once the sludge in stage one was acclimatised.

3.5.1.1 Microbiological composition of sewage

The microbiological composition of sewage has been extensively documented in the literature over the years. Crude sewage contains mainly a large quantity of non-pathogenic microbes as well as a small amount of pathogens that can cause infectious diseases to man (Higgins and Burns, 1975). Members of the harmless bacteria group include *Bacillus subtilis*, *Bacilus megaterium*, *Bacillus mycoides*, *Pseudomonas fluorescens*, *Achromobacter spp.* and *Micrococcus spp.* Bacteria of intestinal origin such as *Escherichia coli*, *Proteus* and *Seratia spp.*, also classified as the Category 2 pathogen are often found in large quantities in sewage. Potential harmful Category 3 pathogens such as *Clostridium perfringens*, enterococci (*Streptococcus faecalis*), *Vibrio cholerae*, *Salmonella typhii*, *Salmonella paratyphii*, *Shigella spp.*, etc. are occasionally present at very low levels.

Activated sludge plants have an established microbial community as they are continuously inoculated by the return of the sludge to the incoming settled sewage. As a result, the habitat or microflora composition is more homogenous and has lower species diversity. The microbial ecology is dominated by organisms which can grow under the prevailing conditions and which either produce a zoogloeal matrix (floc) or which can thrive embedded in the matrix produced by the other bacteria (Burns and Slater, 1982). Bacteria are mostly Gram negative and include members of the genera *Pseudomonas*, *Zoogloea*, *Achromobacter*, *Flavobacterium*, *Nocardia*, *Bdellovibrio*, *Mycobacterium* (Unz and Dondero, 1967) and the nitrifying bacteria, *Nitrobacter* and *Nitrosomonas*. Under conditions of low nitrogen concentration, the following

filamentous genera responsible for causing "bulking" are *Sphaerotilus*, *Nocardia*, *Beggiatoa, Thiothrix, Leucothrix* and *Geotrichum*.

3.5.2 Microscopic examination

Microscopic examination was done with Nikon, Japan, Model Optiphot-2 Microscope and this allowed a close observation on a microscopic level of the protozoa / higher organisms that existed within the membrane bioreactors. The presence of protozoa indicates the existence of a healthy food chain within the MBRs (Curds and Hawkes, 1975). Pictures were also taken with Nikon Coolpix 995 digital camera. Efforts were made to identify the different species of protozoa and the results / pictures are shown in Chapter 6.

3.5.3 Protozoan grazing

Tests were carried out with *Acanthyst amoeba* on the sludge sample from the second stage of the bioreactors. This was to investigate the possibility of protozoan grazing on the sludge with the aim to further reduce sludge production in the second stage. The methodology and results are outlined in Chapter 6.

3.6 Analytical equipment and sample analysis

3.6.1 Biomass determination

The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were determined according to the Standard Methods (APHA, 1975). A glass beaker was dried in the furnace at 550 ± 50 °C for 2 hours to get rid of any volatile compounds. The beaker was allowed to cool in a desiccator and the weight of the empty beaker was noted. Before the sludge sample was taken from the MBR, the aeration was increased to make sure that the content in the MBR was well mixed. 30 ml of sludge was pipetted into the beaker and the beaker was evaporated and dried to constant weight in an oven at 103 – 105 °C. The difference in the weight of the beaker after drying in the oven to when it was empty yielded the MLSS concentration. The residue in the beaker was then dried in a furnace at 550 ± 50 °C for 2 hours. The difference in the weight of the beaker after drying in the furnace to after drying in the oven gave the MLVSS concentration.

3.6.2 Oxygen demand

Oxygen is an important parameter for determining the amount of organic pollution in water. Thus, the following tests were applied for measuring the waste strength of the feed influent stream and for evaluating efficiency of the system with reference to the performance in the effluent discharge streams.

3.6.2.1 Total Organic Carbon (TOC)

The first period of the total organic carbon was measured with a DohrMann DC-180 Carbon Analyzer between Sept'01 and Dec'01. After the commissioning of a new TOC analyser since mid-Dec'01, all TOC measurements were made with a Shimadzu TOC Analyzer Model TOC-5000A. The TOC analyser functions on the principle that the combustion of organic compounds in the combustion tube filled with oxidation catalyst heated to 680°C, releases carbon dioxide (CO_2). The combustion product (CO_2) is then carried by the carrier gas to a sample cell set in a non-dispersive infrared gas analyser (NDIR) where CO_2 is detected. The NDIR outputs a detection signal (analog signal) which generates a peak whose area is calculated by a data processor. The peak area is proportional to the TOC concentration of the sample. A multi-point calibration curve was obtained in advance with potassium hydrogen phthalate ($C_8H_5O_4K$) standard reagent and the relation between the peak area and TOC concentration stored was used for the analysis of TOC concentration in the sample. Figure 3.7 (a) and (b) show the two models of TOC analysers used in this project.

(a) (b)

Figure 3.7: (a) DohrMann DC-180 Carbon Analyzer. (b) Shimadzu TOC Analyzer Model TOC-5000A

3.6.2.2 Biochemical Oxygen Demand (BOD)

The Biochemical Oxygen Demand (BOD) test most closely models aerobic waste treatment and the aquatic ecosystem. It measures oxygen taken up by the microorganisms in the wastewater during the oxidation of organic matter over a five-day period. This is most commonly known as the BOD_5.

The Biochemical Oxygen Demand (BOD) of the system was determined with the WTW OxiTop® Control System (WTW, Germany). The measuring principle of the OxiTop Control system is manometric with a pressure sensor. The BOD bottles are filled according to the BOD measuring range with assigned amounts of water sample. The sample is seeded with a diluted sample of microoganisms from MBR 1 (approximate MLSS concentration of 40 mg/l), placed on an inductive stirring system to ensure good mixing and incubated in an incubator at a temperature of 20 ± 0.5 °C. The microorganisms use oxygen from the air remaining in the closed system to degrade organic substances. The carbon dioxide formed by the process is absorbed by the sodium hydroxide pellet in the rubber quiver above the headspace. The reduction in the amount of oxygen causes the pressure in the bottles to drop. The change in pressure is detected and stored by the piezoelectric measuring head. The data is then transferred to the OC110 Controller via infrared transmission to determine the BOD values. The data can also be downloaded onto a PC program for further analysis via the RS232 connection cable and OCHAT OC software.

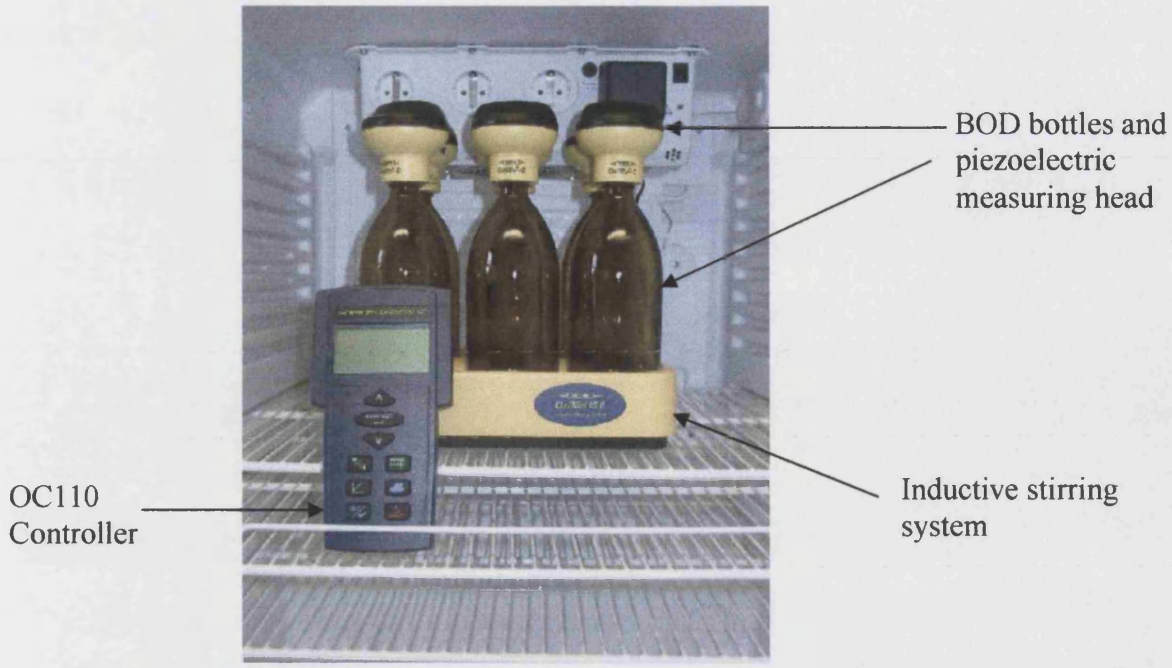


Figure 3.8: WTW OxiTop® Control System, inside the incubator

The procedure for BOD_5 determination is outlined as below (according to WTW OxiTop® manual handbook). The permeate samples (1 litre each) from both MBR 1 and 2 were collected and aerated overnight. Dilution water containing the essential salts was prepared according to the OECD synthetic feed recipe excluding the carbon sources such as meat extract and peptone (refer to the OECD recipe for synthetic sewage feed analogue in the earlier section) and aerated overnight. The dilution water was used as a blank in the test. The sludge from MBR 1 was diluted down a hundred times with dilution water to approximately 40 mg/l and used as an inoculum for the BOD_5 test. 500 ml of permeate sample, with 5 ml of diluted sludge inoculum was mixed together and poured into the 432 ml overflow flask for the BOD range of 0 – 40 mg/l. The content from the overflow flask was then poured into the BOD bottle and the magnetic stirred bar was added. The rubber quiver was placed on top of the bottle and two sodium hydroxide (NaOH) pellets were added into the quiver. The OxiTop® piezoelectric head was screwed tight onto the bottle. The test was initiated with the OC110 Controller and the OxiTop® piezoelectric head automatically stored the data collected. A duplicate was carried out for each of the permeate samples and the blank. The bottles were then placed into the incubator of a constant temperature of 20 ± 0.5 °C. After 5 days of incubation, the data was called out and transferred onto the OC110 Controller, which was then downloaded to a PC where the results could be

analysed. At the same time, the COD and TOC of the permeate samples were analysed in order to find the BOD/COD and COD/TOC ratios for each of the process streams.

3.6.2.3 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) was measured with the Hach's United States Environmental Protection Agency (USEPA) – approved COD method (Hach, USA). Hach's premeasured, ready-to-use reagents contain dichromate ($K_2Cr_2O_7$) standard solution, sulphuric acid and a strong oxidant with a silver compound added as a catalyst to promote oxidation of resistant organic compounds. Mercuric sulfate is also included to reduce interference from the oxidation of chloride ions by dichromate. The digestion is carried out with Hach's COD Reactor at a temperature of 150 °C. The reagent vial necks and caps reach a temperature of approximately 85 °C because they extend above the reactor. The temperature differential between the top and bottom of the vial induces proper refluxing action. Hence, Hach COD test replaces the cumbersome open reflux set-ups. The COD of the samples after digestion can be read off using the Hach DR/890 Colorimeter.

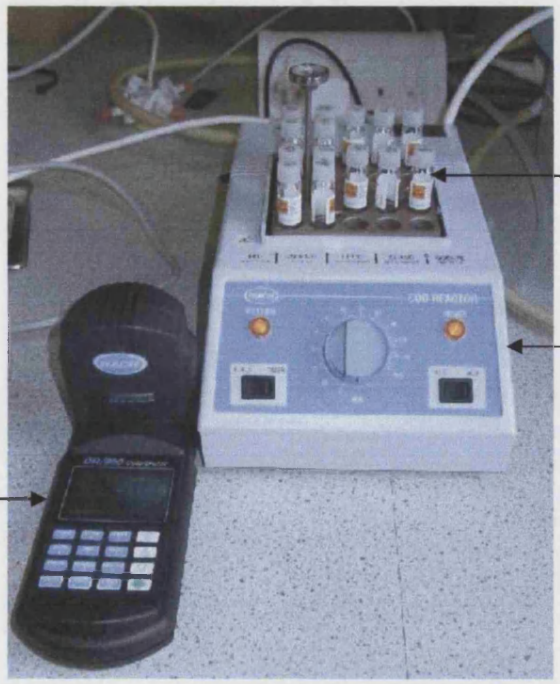


Figure 3.9: Hach's COD Reactor and Hach DR/890 Colorimeter

The procedure for COD tests (according to Hach's COD manual handbook) was as follows: 2 ml of sample is pipetted into the screw-cap reagent vials and the cap was replaced tightly. The COD reagent vials come in two ranges, the High Range 0 – 1500 mg/L and the Low Range 0 – 150 mg/L. Both the permeate from MBR 1 and 2 were analysed with the Low Range COD vials while the concentrated feed was diluted down by one tenth of its concentration and analysed with the High Range COD vials. The COD reactor was preheated to 150 °C. The reagent vials were inverted gently several times to mix the content and placed in the preheated COD Reactor for 2 hours. A blank was also prepared in the same way, but with 2 ml of deionized water. After 2 hours digestion, the vials were inverted several times while still warm and removed to a rack until they cooled to room temperature. The outside of the reagent vials was cleaned with a towel. The results were read off using the DR/890 Colorimeter at the appropriate pre-programmed and stored wavelength for each of the COD ranges. The blank was used as a zero point for the reading of other samples.

3.6.3 Ammonia, Nitrate and Nitrite Analysis

Hach's USEPA Test 'N Tube and Hach's DR/890 Colorimeter (Hach, USA) are used for the analysis of ammonia (NH_3), nitrate (NO_3^-) and nitrite (NO_2^-) ions. Hach's Test 'N Tube™ Analysis Systems is designed for maximum efficiency and minimal waste featuring premeasured reagents – in the vial or available in sealed, unit-dose packages. The results can be read off directly from the Hach's DR/890 Colorimeter after the reaction time.

3.6.3.1 Ammonia

Hach Test 'N Tube ammonia nitrogen, high range (0 – 50 mg/L NH_3-N) reagent vials were used for the analysis of ammonia nitrogen (NH_3). The procedure for the ammonia test (according to Hach's Test 'N Tube manual handbook) was as follows: The stored programme number was entered into the Hach DR/890 Colorimeter (Program 67). 0.1 ml of permeate sample was pipetted to the AmVer Diluent Reagent High Range Vial, while 0.1 ml of deionized water (provided in the test kit) was added to another vial (this served as the blank). The contents of one Ammonia Salicylate Reagent Powder Pillow were added to each of the two vials, followed by the addition of one Ammonia Cyanurate Reagent Powder Pillow to each vial. The vials were

tightly capped and shaken thoroughly in an inverted motion. A green colour will develop if ammonia is present. Then, the timer on the Hach DR/890 Colorimeter was activated and a twenty-minute reaction period will begin. The outside of the vial was cleaned with a towel (to remove fingerprints and other marks) and when the timer beeps, the blank was placed into the vial adapter of the Hach DR/890 Colorimeter and the instrument cap was placed over the vial. The ZERO button was pressed. Then, the sample test vial was placed in the adapter, covered tightly with the cap and with the READ button pressed, the results were displayed in mg/L NH_3-N.

3.6.3.2 Nitrate

Hach Test 'N Tube nitrate nitrogen, high range (0 – 30 mg/L NO_3^--N) reagent vials were used for the analysis of nitrate nitrogen (NO_3^-). The procedure for the nitrate test (according to Hach's Test 'N Tube manual handbook) was as follows: The stored programme number was entered into the Hach DR/890 Colorimeter (Program 57). 1 ml of permeate sample was pipetted to a Nitrate Pretreatment Solution vial (the blank). The vial was tightly capped and inverted 10 times to mix. The outside of the vial was cleaned with a towel (to remove fingerprints and other marks) and the vial was placed into the vial adapter of the Hach DR/890 Colorimeter, covered tightly by the instrument cap over the vial. The ZERO button was pressed. Then, the contents of one NitraVer X Reagent B Powder Pillow were added to the vial. The vial was inverted 10 times to mix (this will be the prepared sample). A yellow colour will develop if nitrate nitrogen is present in the test sample. Then, the timer on the Hach DR/890 Colorimeter was activated and a five-minute reaction period will begin. After the timer beeps, the outside of the vial was cleaned and placed in the adapter, covered tightly with the cap. With the READ button pressed, the results were displayed in mg/L NO_3^--N. For most accurate results, a Reagent Blank Correction was performed previously with 1 ml of deionized water, following the same test procedure.

3.6.3.3 Nitrite

Hach Test 'N Tube nitrite nitrogen, low range (0 – 0.5 mg/L NO_2^--N) reagent vials were used for the analysis of nitrite nitrogen (NO_2^-). The procedure for the nitrite test was as follows: The stored programme number was entered into the Hach DR/890 Colorimeter (Program 63). 5 ml of permeate sample was pipetted to a NitriVer vial and the vial was capped and shaken to dissolve powder (this was the prepared

sample). Then, the timer on the Hach DR/890 Colorimeter was activated and a twenty-minute reaction period will begin. A pink colour will develop if nitrite is present. Meanwhile, an empty Test 'N Tube vial was filled with 5 ml of permeate sample (the blank). The outside of the vial (the blank) was cleaned with a towel (to remove fingerprints and other marks) and the blank was placed into the vial adapter of the Hach DR/890 Colorimeter, covered tightly by the instrument cap over the vial. The ZERO button was pressed. After the timer beeps, the outside of the prepared sample vial was cleaned and placed in the adapter, covered tightly with the cap. With the READ button pressed, the results were displayed in mg/L NO_2^--N.

The permeate samples from MBR 1 and 2 were diluted down to a range within the measurement range of the nitrogen tests. The final results are then obtained by adjusting with the dilution factor.

3.6.4 Respirometry

The OxiTop® Control System was also used for respirometry in order to find the kinetics of the MBRs. The data recorded by the piezoelectric pressure head was converted into the cumulative oxygen uptake rate and used to calculate the growth kinetics of the microbial system. Chapter 5 Respirometry as a Tool for Determination of Kinetic Parameters of MBR 1 and MBR 2 outlines the background theory and literature review of respirometry. Readers are referred to Chapter 5 also for the complete procedures and methodology as well as experimental results and discussion for the kinetics of MBR 1 and MBR 2.

3.7 New MBR 2

A new MBR 2 was set up to investigate the start up trend of the original MBR 2 due to the disturbance of the algae bloom in the original MBR 2 at the start up. The new MBR 2 was of a smaller scale than the original MBR 2 (1 L compared to 3.5 L) in order that the system would reach steady state in a shorter time. A Millipore crossflow membrane module was used with a 0.45 μm polymeric Millipore membrane. In order to maintain the culture at a constant temperature of 20 – 22 °C, the 2-Litre flask was immersed into a water bath. A thermometer was used to monitor the temperature in the reactor. UNIPROBE P2 polarographic dissolved oxygen electrode and Model 502

DO meter were used for monitoring and maintaining the dissolved oxygen concentration of the system above 2 mg/l, by adjusting the air flowrate. pH was monitored using an autoclavable, Ingold combined pH electrode (with compensation for temperature, range 0 – 130°C). A Watson-Marlow 501U peristaltic pump was used to pump the mixed liquor to the external microfiltration (MF) unit. Figure 3.10 and 3.11 show the schematic of the system and the set up of the new MBR 2 in operation.

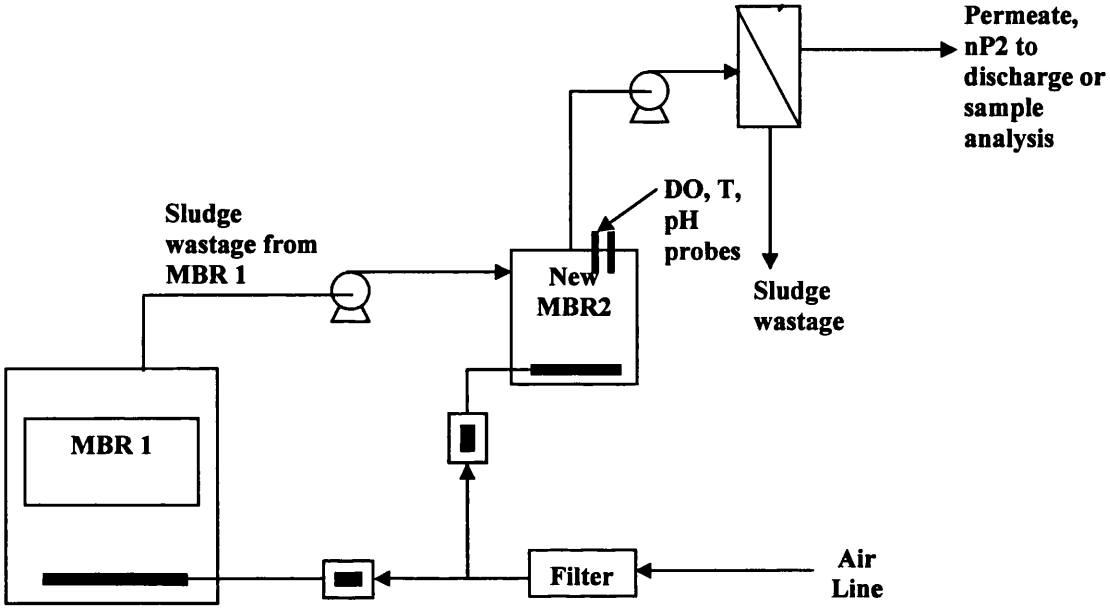
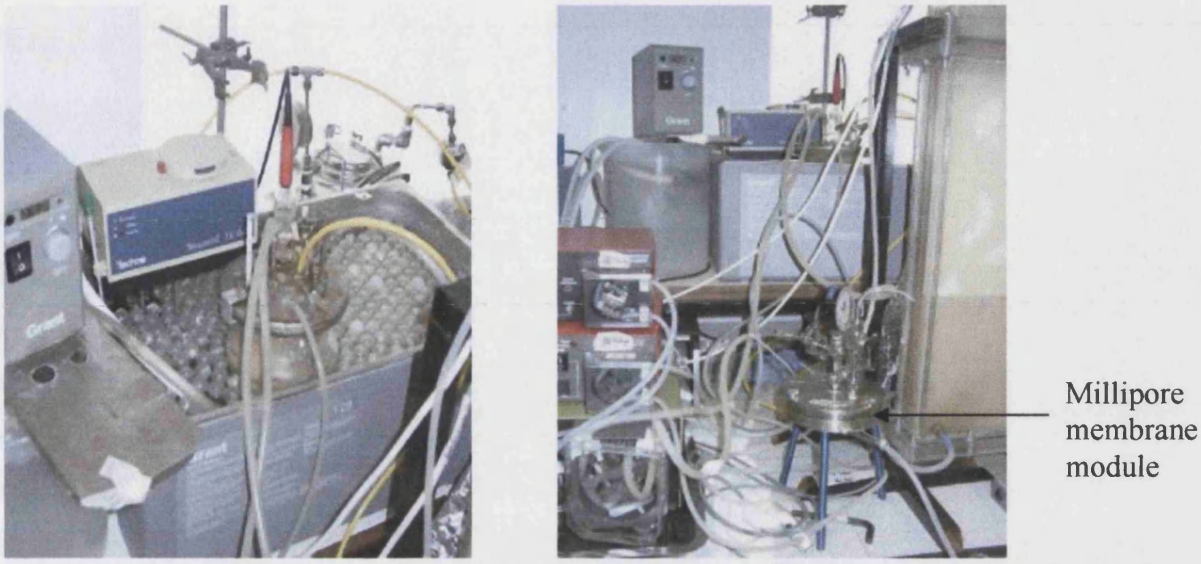


Figure 3.10: Schematic diagram for the new MBR 2



(a) (b)

Figure 3.11: (a) New MBR 2 immersed in the water bath. (b) The external Millipore membrane module.

Chapter 4: Performance of the Membrane Bioreactor

Scope of Chapter 4

In this chapter, the differences between the two membrane bioreactors are presented and compared in terms of performance as seen from the experimental results. The performance of both the MBRs in terms of permeate discharge quality (Biochemical Oxygen Demand and Chemical Oxygen Demand), BOD and COD removal rate, sludge yield, volumetric sludge production and nitrogen concentration are reported and compared with the literature. The experimental objectives were made clear in the previous chapter and the different stages of the MBR played a specific role in achieving the objective of the project. The 1st stage was focused on maximal organic degradation, while the 2nd stage had its emphasis on reducing the excess sludge production. Refer to Chapter 3 for the operating conditions of the two-stage MBR. The transitions of the MBRs from start-up into steady state condition were observed throughout a period of more than a year (424 days of analytical period). A new 2nd stage MBR was set up to reinvestigate the initial development, as the original MBR 2 had undergone disturbance from algal bloom at the early stage.

4.1 Start-up of the MBRs

Two membrane bioreactor (MBR) experimental rigs constructed of Perspex, each of a working volume of 3.5 L were completed and fully commissioned by May'01. The MBR stage 1 (MBR 1) was seeded with activated sludge sample collected from the secondary activated sludge tank from the sewage treatment plants in Cam Valley STW in April'01. This activated sludge seed had a MLSS concentration of approximately 2,500 – 3,000 mg/l. The sludge in MBR 1 was allowed to slowly acclimatized to the synthetic feed according to a derived recipe in an OECD Technical Report (1976) on surfactant biodegradability. There was no sludge wastage during the acclimatization period in order to build up the biomass concentration. The MLSS of MBR 1 increased gradually and reached a value of 9,200 mg/l after 2 months' operation. At this point, MBR stage 2 (MBR 2) was started up by receiving a daily amount of 218.75 ml of sludge wasted from the first stage at a SRT of 16 days. After wasting sludge at a SRT of 16 d for a month, the MLSS of MBR 1 dropped rapidly to 4,700 mg/l by the end of July'01.

At the end of July'01, the experimental rigs were shut down and moved over to the new building. The rigs were started up again in Aug'01. MBR 1 slowly reached a steady state under a HRT of 6 h and a SRT of 16 d, while MBR 2 started filling up with an infinite HRT and SRT. The analytical work started from the beginning of Sept'01 over a period of 424 days and the results are presented in the following sections.

4.2 Performance of MBRs from start-up into steady state

The discharge permeate quality from MBR 1 and MBR 2 has to meet stringent legislative regulation set by the regulatory bodies. In the following section, the performance of the permeate streams of both MBRs has been assessed and analysed and parameters such as COD, BOD, COD and BOD removal rates are monitored over a period of 424 days. Other parameters monitored are sludge yield, volumetric sludge reduction, volumetric COD removal rate, SOUR and evolution of biomass from start-up into steady state.

4.2.1 Chemical Oxygen Demand (COD)

The analysis was carried out with a Shimadzu TOC Analyzer Model TOC-5000A in conjunction with the use of Hach COD analysis (Hach, USA). After both MBRs had reached steady states, the COD/TOC ratio for the feed medium, permeate of MBR 1 and MBR 2 were determined experimentally in order to calculate the COD values (see Table 4.1). The COD/TOC ratio is useful for characterizing the different streams as the Hach COD vial tests are more expensive than the TOC analysis (if a TOC analyzer is readily available).

Table 4.1: COD/TOC ratio for different streams in the MBR system at steady state.

Stream	COD/TOC ratio
Feed	2.65 ± 0.04
Permeate of MBR 1	3.97 ± 0.46
Permeate of MBR 2	2.96 ± 0.39

4.2.1.1 COD of the concentrated feed

The synthetic feed was prepared according to the OECD recipe as defined by the MBR-T committee, to give a feed strength of 500 mg COD/l (see Chapter 3). The OECD recipe was selected to represent the settled sewage after primary settling. Typical COD strength for untreated domestic wastewater varied between 250 mg COD/l (weak) to 1,000 mg COD/l (strong) with a medium value of 500 mg COD/l (Metcalf and Eddy, 1991). However, it can be seen from Figure 4.1 that the COD value of the feed input was lower than the expected value. During the analytical period between Day 1 of analysis and Day 79, the average value of the feed varied between 300 – 350 mg COD/l. This was when the TOC values were measured with the old DohrMann DC-180 Carbon Analyzer. Due to the breakdown of the TOC analyzer from Day 81 to Day 98, measurement could not be made. A new Shimadzu TOC Analyzer Model TOC-5000A was commissioned on Day 99 and the feed strength since then was found to be between 350-400 mg COD/l. It could be concluded that there was a discrepancy in the results between the two TOC analyzers. The discrepancy could be due to the inaccuracy of the old DohrMann DC-180 Carbon Analyzer compared to the new Shimadzu TOC Analyzer Model TOC-5000A. The inconsistency in preparing the feed stock at the earlier stages could also have an effect on this discrepancy. More consistency in the feed stock preparation was reflected by the less variability or noise in the measured results between Day 121 and Day 403. The average COD value of the feed starting from Day 124 to Day 403 was measured to be 404.9 ± 21.9 mg COD/l.

There were also a few instances where the feed strength had dropped below 300 mg COD/l. This was due to the possible contamination of this highly concentrated rich nutrient as it was prepared in 10-times concentrated solution. However, it can be seen from Figures 4.1 and 4.2 that overall the fluctuations in the feed strength did not cause a big disturbance to the permeate quality or the removal efficiency in MBR 1.

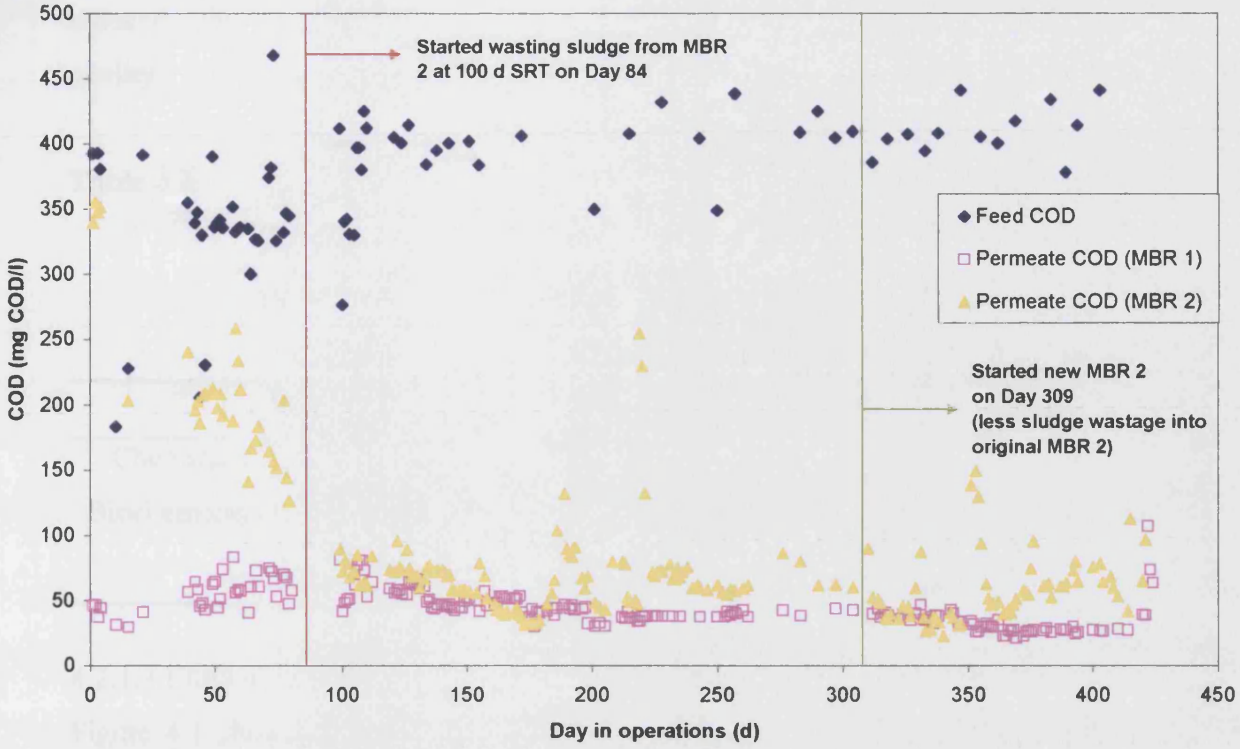


Figure 4.1: COD of synthetic feed, permeate of MBR 1 and of MBR 2 against time (Operating conditions of MBR 1: 6 h HRT, 16 d SRT; MBR 2: 19 d HRT, 100 d SRT)

4.2.1.2 COD of permeate in MBR 1

As observed from Figure 4.1, since Day 1 of the analytical period, the permeate quality of MBR 1 appeared to vary between a range of 32 to 83 mg COD/l. Starting from Day 172, analysis of the trend indicates that the permeate of MBR 1 reached an average steady state value of 39.1 ± 3.7 mg COD/l, which is lower than the discharge consent of 125 mg COD/l (see Table 4.2). The BOD of the permeate was also satisfactorily within the discharge limit, as shown in Section 4.2.2. Table 4.2 shows the discharge limits and the minimum % reduction required by the Urban Waste Water Treatment Directive (91/271/EEC) for the effluent standards of secondary sewage treatment (Gray, 1999). Starting from Day 332, the permeate quality was seen to further improve and the average COD calculated was 27.4 ± 2.7 mg COD/l. The results showed that the system is changing and finally reaching a steady state with satisfactory permeate quality after a long period of time, in this case, after more than a year's time (424 days), where the noise in the measured permeate COD values had

reduced. The permeate of MBR 1 was discharged straightaway due to its excellent quality.

Table 4.2: Requirements for discharges from secondary sewage treatment plants under the Urban Waste Water Treatment Directive (91/271/EEC) – (Gray, 1999)

Parameter	Discharge limits (mg/l)	Minimum % reduction
Chemical Oxygen Demand (COD)	125	75
Biochemical Oxygen Demand (BOD_5)	25	70-90
Suspended Solids	35	90

4.2.1.3 COD of permeate in MBR 2

Figure 4.1 shows that the permeate quality in MBR 2 started off with a very high value of around 340 mg COD/l since Day 1 of the analytical period. This was possibly due to the initial breakdown of the cells and hence the release of soluble lysis products, however, this value dropped gradually with time. By Day 79, four months into operation since Aug'01, this value decreased to 127 mg COD/l. It is proposed that the improvement in the permeate quality was possibly due to the gradual stabilization of the system.

Starting from Day 84, MBR 2 was operated at a SRT of 100 d and HRT of 19 d. A relatively much higher SRT was chosen for MBR 2 compared to MBR 1 because higher SRTs are associated with lesser sludge production. However, within the experimental timeframe that was available (1 year), a 100 d SRT was more realistic for MBR 2 to achieving steady state instead of a much higher SRT. As a result of sludge wastage, a further improvement in the permeate quality can be seen from Figure 4.1. Between the period of Day 99 and Day 179, the permeate COD gradually decreased from a value of 89 mg COD/l to as low as 36 mg COD/l. The drop in the permeate COD value since sludge started to be wasted from MBR 2 may be attributed to the wash out of bacteria before there was time for lysis. This was later confirmed from the results shown in Figure 4.1, between Day 182 and Day 205. During this period, sludge wastage from MBR 2 was not being carried out. It could be seen that

the COD value had increased to 120 mg COD/l on Day 189. However, the permeate quality had improved gradually when sludge was being wasted again from MBR 2 at a SRT of 100 d.

MBR 2 were only receiving sludge wasted from MBR 1, hence the readily soluble substrate into MBR 2 was relatively much lower compared to MBR 1 with a regular easily degradable substrate supply with a feed strength of 400 mg COD/l. Therefore, in MBR 2, the effect of the endogenous coefficient, k_d was more significant in comparison to the maximum specific growth rate, μ_m. This means that the cells are lysing or decaying at a faster rate than growth due to the lack of readily degradable substrate. It is well known from the literature that a longer SRT is associated with a reduced biosludge production (Henze *et al.*, 1987; Saunamaki, 1988), which in turn may be attributed to a much bigger k_d. Hence, this effect could explain the higher soluble COD value in the permeate when MBR 2 started off with an infinite SRT. When there was no sludge wastage, k_d was significant and the cells were lysing and hence the lysates contributed towards an increase in the soluble COD of the permeate. When the SRT was dropped from infinity to 100 d, k_d decreased and some of the cells were washed out before they have more time to lyse. This would then explain the gradual improvement in the permeate quality at a SRT of 100 d. In theory, there would be a limitation in the biomass that could be supported in the MBR before the build-up of inorganic salts that leads to an unacceptable COD value in the permeate. It is proposed that an optimum sludge age may exist where the COD value is within the discharge consent and yet there is also lesser excess sludge production.

There was a shock increase in the COD up to 255 mg COD/l on Day 219. This could be explained from the addition of a few drops of 1 M of sodium hydroxide (NaOH) to try to rectify the acidity of the system (as discussed later). However, Rocher *et al.* (1999) reported using NaOH as an agent to promote cell lysis and this explained the sudden increase in COD value. After the impact of the NaOH was over, the COD of the permeate eventually dropped and fluctuated between 60 and 77 mg COD/l. From Day 240 to Day 319, (before the startup of a new MBR 2), a further improvement in the permeate quality could be observed where an average steady state value of 58.8 ± 15.0 mg COD/l was recorded. It is proposed that MBR 2 has reached steady state due

to the lesser noise in the COD measurements. During the 424 days of analytical period, some scatter of the data could be noticed due to possible errors in analytical measurements.

4.2.1.4 COD removal rate in MBR 1

Figure 4.2 shows that the COD removal rate for MBR 1 fluctuated between 80 and 90% since Day 1. There were a few occasions where this was lower than 80%, but always above 75 %. The noise was due to the scattering of feed COD measurements using the old DohrMann DC-180 Carbon Analyzer and the transition of MBR 1 from start-up to approaching steady state.

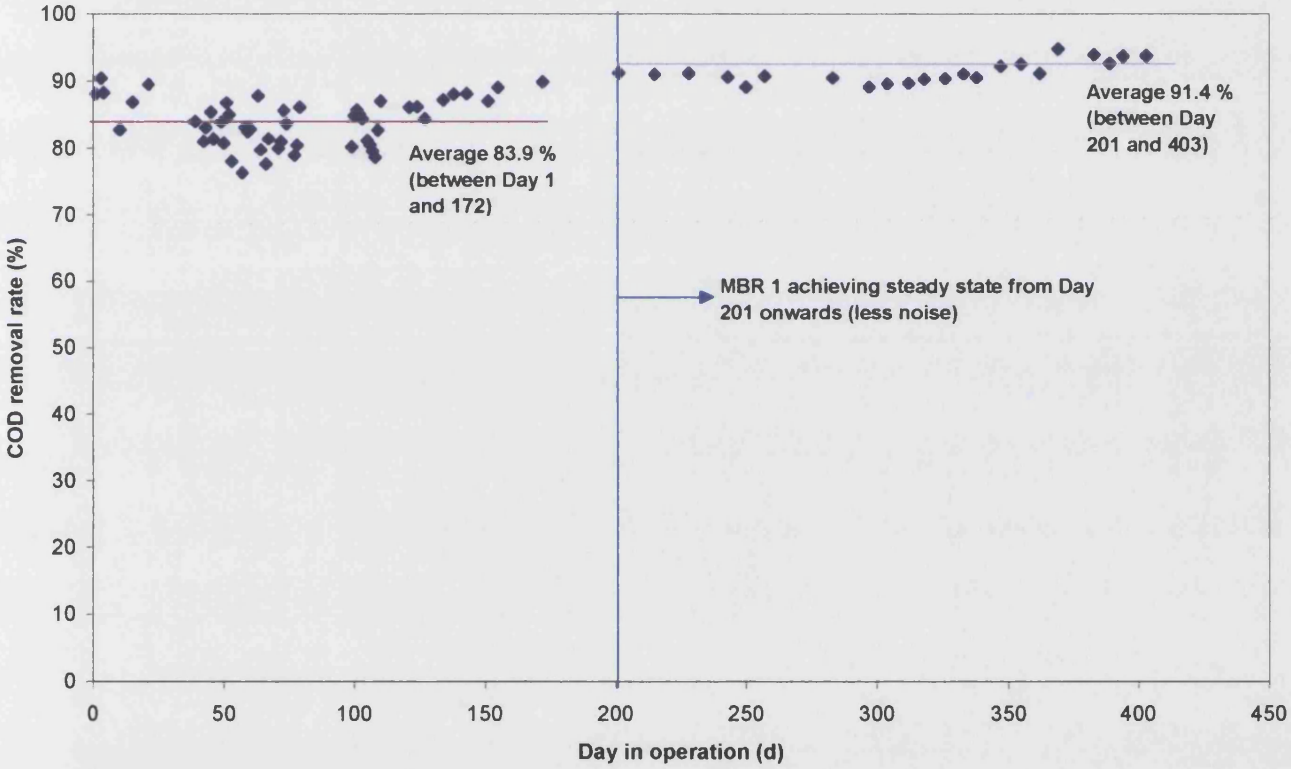


Figure 4.2: COD removal rate (%) of MBR 1 against time (Operating conditions of MBR 1: 6 h HRT, 16d SRT)

Between Day 1 and Day 172, the average COD removal rate was 83.9 ± 3.5 %. From Day 172 onwards, the COD removal efficiency of the system as well as the permeate of MBR 1 (refer to Figure 4.1) had seen a further improvement. This can be seen from the lesser degree of noise level in the data measurements and the new average COD removal rate after the system had reached steady state from Day 201 onwards to Day

403 was 91.4 ± 1.6 %. The excellent COD removal rate was well above the minimum COD reduction of 75% required to meet the legislative regulation for discharges from secondary sewage treatment plants under the Urban Waste Water Treatment Directive (91/271/EEC) – see Table 4.2 (Gray, 1999).

4.2.2 Biological Oxygen Demand (BOD_5) and BOD_5 removal rate

OxiTop® Control System, (WTW, Germany) was used to determine the Biochemical Oxygen Demand (BOD_5) of the system. Refer to Chapter 3 for the measuring principles of OxiTop®, as well as the procedures for BOD_5 measurement. As well as determining the BOD_5 values for the different streams, COD values were measured to enable the determination of the BOD/ COD ratios for the different streams (see Table 4.4). The results of the BOD_5 tests for the synthetic feed, permeate of MBR 1 and permeate of MBR 2 are tabulated in Table 4.3.

Table 4.3: BOD_5 values of synthetic feed, permeate of MBR 1 (P1) and permeate of MBR 2 (P2).

Run No.	Synthetic Feed (mg BOD_5/l)	Permeate of MBR 1 (mg BOD_5/l)	Permeate of MBR 2 (mg BOD_5/l)
1	228.2	4.5	15.0*
2	253.4	3.6	10.3*
3	273.4	2.8	11.2
4	-	4.7	-
5	-	5.0	-
6	-	3.7	-
7	-	4.2	-
Mean ± std. Dev.	251.7 ± 22.6	4.1 ± 0.8	12.2 ± 2.5

** Results had been calculated using a BOD/COD ratio of 0.174, which was obtained from the only set of reasonable measured results in Run 3.*

The synthetic feed showed relatively consistent results with an average value of 251.7 ± 22.6 mg BOD_5/l. Within the literature, typical BOD_5 strength for untreated domestic wastewater varied between 110 mg BOD_5/l (weak) to 400mg BOD_5/l (strong) with a medium value of 220 mg BOD_5/l (Metcalf and Eddy, 1991). Therefore, the BOD_5 value of the synthetic feed corresponded well to the medium value reported in the

literature. The permeate quality of MBR 1 was very good with an average value of 4.1 ± 0.8 mg BOD_5/l, well below the current discharge limit of 25 mg BOD_5/l (see Table 4.2). The scattering of the BOD_5 results corresponded to the fluctuating measured COD values in the permeate.

There was difficulty in measuring the BOD_5 values for permeate of MBR 2, and only one set of measured result seemed reasonable, where the BOD_5 was 11.2 mg BOD_5/l corresponding to a COD of 64.3 mg COD/l, giving a calculated BOD/ COD ratio of 0.174. This was due to the time and equipment limitations as only six BOD bottles were available at any time, with three streams to investigate. Moreover, the nature of permeate of MBR 2 (remembering there was no constant supply of easily degradable feed and the system focusing on sludge digestion) made it difficult to determine the BOD_5 values in permeate of MBR 2. Therefore, the other two BOD_5 values for permeate of MBR 2 were calculated from the measured COD values in the permeate, based on the BOD/COD ratio of 0.174 (the only reasonable set of results). If the BOD_5 results measured for MBR 2 are trustworthy, the BOD_5 value in permeate of MBR 2 (12.2 mg BOD_5/l) is still below the current discharge limit of 25 mg BOD_5/l (see Table 4.2).

As mentioned before, COD values in the different streams were also measured at the time when BOD_5 values were determined, and the calculated BOD_5/COD ratio for the synthetic feed, permeate of MBR 1 and MBR 2 are tabulated in Table 4.4. The correlation between COD and BOD can be very useful because the COD can be determined in three hours, compared with five days for the BOD. Therefore, once the correlation has been established, COD measurements can be used to good advantage for treatment plant control and operation (Metcalf and Eddy, 1991). The index indicates how easily biodegradable is a stream by microorganisms in a system. The easily biodegradable nature of the synthetic feed stream is clearly depicted by the higher BOD_5/COD ratio, whereas in permeate of MBR 1 and MBR 2, the lower values show a less biodegradable nature as the easily biodegradable substrate was degraded by the microorganisms in MBR 1 and MBR 2. The calculated BOD_5/COD ratio for the synthetic feed of 0.6 also falls within the range reported in the literature for typical untreated domestic wastewater, which varies from 0.4 to 0.8 (Metcalf and Eddy, 1991).

Table 4.4: BOD_5/COD ratio for different streams in the MBR system.

Stream	BOD_5/COD ratio
Feed	0.600 ± 0.05
Permeate of MBR 1	0.117 ± 0.03
Permeate of MBR 2	0.174

Based on the average BOD_5 results for the synthetic feed and permeate of MBR 1, the calculated BOD removal rate in MBR 1 mounted up to approximately 98%, which is higher than the minimum BOD reduction of 70 – 90% required by the legislation (see Table 4.2).

4.2.3 Evolution of biomass

4.2.3.1 MBR 1

Figure 4.3 shows that the biomass in MBR 1 slowly acclimatized to the synthetic sewage recipe for the operating conditions of 6h HRT and 16 d SRT. Starting Day 1 of the analytical period, the mixed liquor suspended solids (MLSS) value was 3,990 mg/l and the mixed liquor volatile suspended solids (MLVSS) value was 3,550 mg/l. The biomass increased gradually up to a maximum value of 6,100 and 5,500 mg/l respectively by Day 96. Since then, it can be seen that the biomass fluctuated between 5,000 and 6,000 mg/l. There are a few cyclic waves in which the system was approaching steady state from start-up and due to human errors in measuring MLSS and MLVSS values. During the period between Day 201 and Day 291, MBR 1 had an average MLSS of 4,580 ± 150 mg/l and MLVSS value of 4,220 ± 140 mg/l. These values increased since Day 299 and the new average steady state values from Day 299 to Day 424 are 5,280 ± 420 mg/l and 4,920 ± 350 mg/l for MLSS and MLVSS respectively. The MLVSS/MLSS ratio fluctuated between 87 – 96 %, which shows that the sludge in MBR 1 was mostly organic solids and there was very little inorganic present due to the easily degradable nature of the synthetic feed. The biomass was more viable and actively reproducing (cell replication) in MBR 1 than in MBR 2 due to a shorter SRT. This was later confirmed by the presence of a larger quantity of higher organisms in MBR 1 than in MBR 2 from the microscopic examinations (see Chapter 6). As reported in the literature, the higher organisms such as ciliated

protozoa and metazoa feed on viable bacteria in the wastewater treatment plants (Lee and Welander, 1996; Rensink and Rulkens, 1997; Ghyoot and Verstraete, 1999).

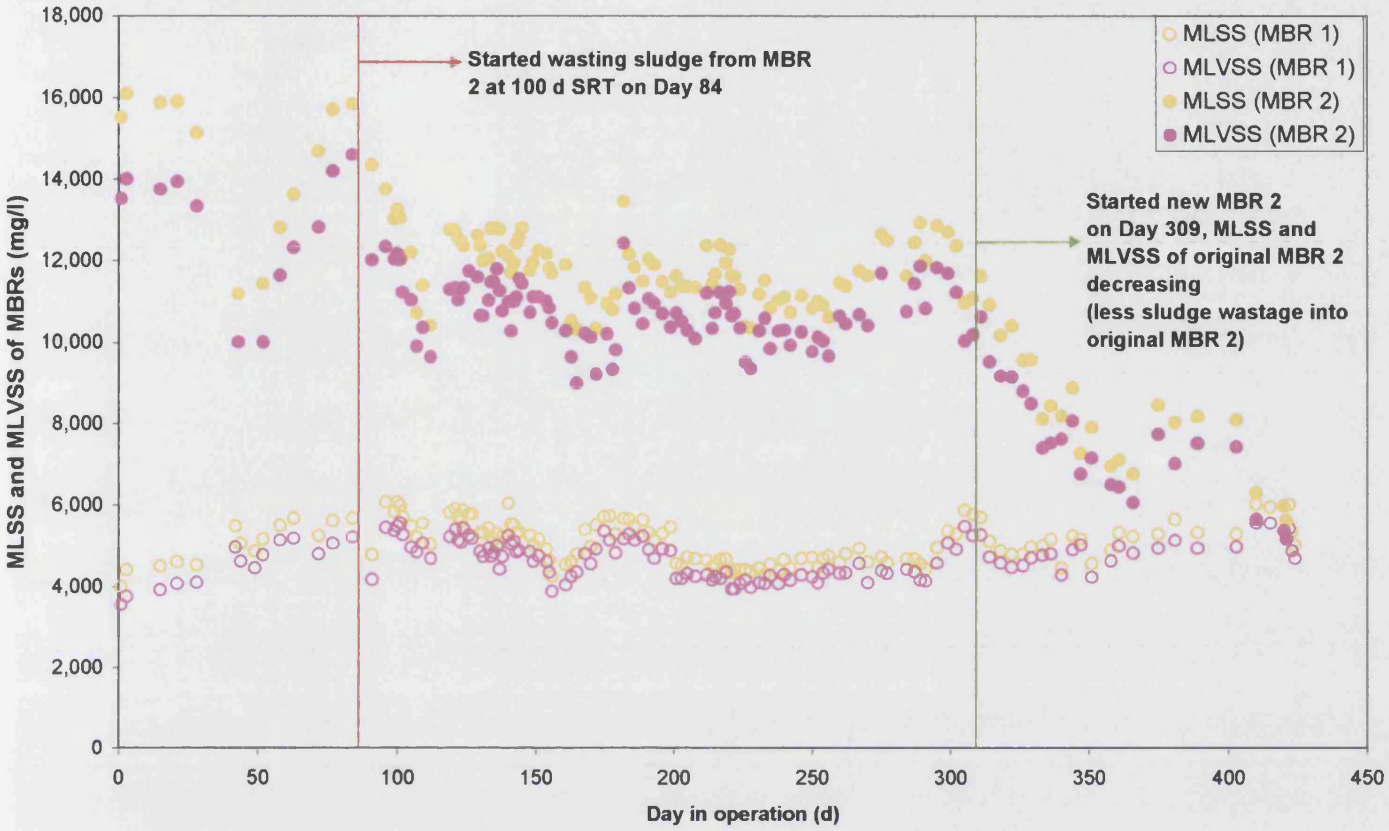


Figure 4.3: MLSS and MLVSS of MBRs against time (Operating conditions of MBR 1: 6 h HRT, 16 d SRT; MBR 2: 19 d HRT, 100 d SRT)

4.2.3.2 MBR 2

After the start-up of MBR 2 in Jul'01, it was operated with no sludge or permeate withdrawal. The MLSS and MLVSS values were 6,470 mg/l and 5,940 mg/l respectively at start-up. These gradually increased up to 12,620 mg/l and 11,010 mg/l after 2 months' operation in Aug'01. It can be seen from Figure 4.3 that starting from Day 1 of the analytical period (Sep'01), the values had further increased up to 16,100 mg/l and 14,010 mg/l. Oscillations in the development of the biomass can be seen between Day 28 and Day 84, due to the system experiencing some instability as a result of the absence of accurate level control. The MLSS and MLVSS dropped to 11,200 mg/l and 10,000 mg/l on Day 43 and increased back to 15,850 mg/l and 14,600 mg/l on Day 84. Starting from Day 84, sludge was wasted from MBR 2 at a SRT of 100 days due to algal bloom. The effect of sludge wastage resulted in the

rapid drop of the MLSS concentration from a value of 15,850 mg/l to an average steady value around 13,000 mg/l on Day 119.

There were a few incidents with the failure of the level control, which led to the fluctuations in the biomass development. For instance, on Day 105, the level control in MBR 2 failed and the level increased due to the addition of water into the system, causing a dilution of the biomass. This failure repeated on Day 107, and the disturbance to the system can be seen from the drop in biomass between the period of Day 105 and Day 112. Since then, MBR 2 slowly stabilized and the MLSS and MLVSS values increased back to 12,760 mg/l and 11,290 mg/l on Day 119. For the period between Day 119 and Day 308, the MLSS and MLVSS had established itself to average values of 11,770 ± 730 and 10,670 ± 680 mg/l. It can be concluded that the system had reached steady state, not taking into account a few occasions where disturbances occurred. Since the start-up of the new MBR 2 on Day 309, it could be seen that the biomass in the original MBR 2 decreased gradually, due to the reduction in sludge wastage from MBR 1 into the original MBR 2. The MLVSS/MLSS ratio varied between 85 – 93 %. This showed that the sludge in MBR 2 is also mostly organic solids and there was very little build-up of inorganics. However, sludge is less viable (cell lysis is greater than cell replication) in MBR 2 due to the much longer SRT. This was later confirmed with the relatively less higher organisms found in MBR 2 compared to MBR 1 from the microscopic examinations (see Chapter 6).

4.2.4 Observation and microscopic examination of the sludge

4.2.4.1 MBR 1

The sludge in MBR 1 had a yellowish / orange colour. Microscopic examination showed the presence of ciliated protozoa, rotifers and nematodes – see Chapter 6 for microscopic evidence of this.

4.2.4.2 MBR 2

Over time, the sludge in MBR 2 turned green. Microscopic examination confirmed the presence of algae and cyanobacteria. To combat this, MBR 2 was shielded from sunlight with aluminium foil, and sludge was wasted at 100 d SRT on Day 84 in order to try and wash out these photosynthetic organisms. After approximately 60 days, it

could be seen from the colour change of the sludge from green to dark brown that the algae had been washed out from the system. During Day 127 – 133 (when tests were carried out with amoeba – see Chapter 6), higher organisms were observed. However, due to nitrification and the gradual pH drop (from an initial pH of 7.0 on Day 1 of the analytical period to 3.8 on Day 240) in MBR 2, the higher organisms slowly disappeared as observed by the microscopic examinations (pictures to be found in Chapter 6).

4.2.5 Sludge yield and sludge production of MBRs

4.2.5.1 MBR 1

The sludge yield and sludge production rate of MBR 1 varied during the start up. As the biomass acclimatized to the synthetic feed, the yield value increased together with the increase in MLSS. Figure 4.4 shows that it varied between 0.17 and 0.40 g MLSS/g COD and the average value (calculated from Day 203 to Day 415) is 0.20 ± 0.02 g MLSS/g COD. The sludge production rate is variable between 0.87 and 1.33 g MLSS/d with a mean value of 1.06 ± 0.09 g MLSS/d.

4.2.5.2 MBR 2

Following start up, the sludge yield and sludge production rate of MBR 2 also went through oscillation during acclimatization. Figure 4.4 clearly shows a big reduction in both sludge yield and sludge production of MBR 2 in comparison to MBR 1. Between Day 1 of the analytical period and Day 305 (before the start-up of the new MBR 2 on Day 309), the sludge yield varied between 0.23 g MLSS/g COD and 0.10 g MLSS/g COD. This shows that cell lysis and endogenous metabolism were more dominant in MBR 2. MBR 2 was operated at a low average F/M ratio or sludge loading of 0.037 ± 0.005 g COD/g MLSS/d, as sludge wasted from MBR 1 was used as a source of substrate. This is much lower compared to the F/M ratio of 0.35 g COD/g MLSS/d in MBR 1. Therefore, the growth rate is much lower in MBR 2, leading to a reduction of excess sludge production. Also, the phenomenon of "cryptic" growth may well be occurring, where the biomass grow on their lysis products (Ryan, 1959; Mason and Hamer, 1987; Liu and Tay, 2001). This resulted in the reuse of the carbon source over and over again, leading to a reduced sludge production in terms of the overall carbon balance. Between Day 121 and Day 302 (before the start-up of the new MBR 2 on

Day 309), the yield reached a steady state, averaging 0.14 ± 0.01 g MLSS/g COD. The sludge production rate in MBR 2 followed the same trend of development of the sludge yield values. It started with a value of 0.54 g MLSS/d on Day 1 of the analytical period and decreased to an average value of 0.41 ± 0.03 g MLSS/d between Day 121 and Day 302.

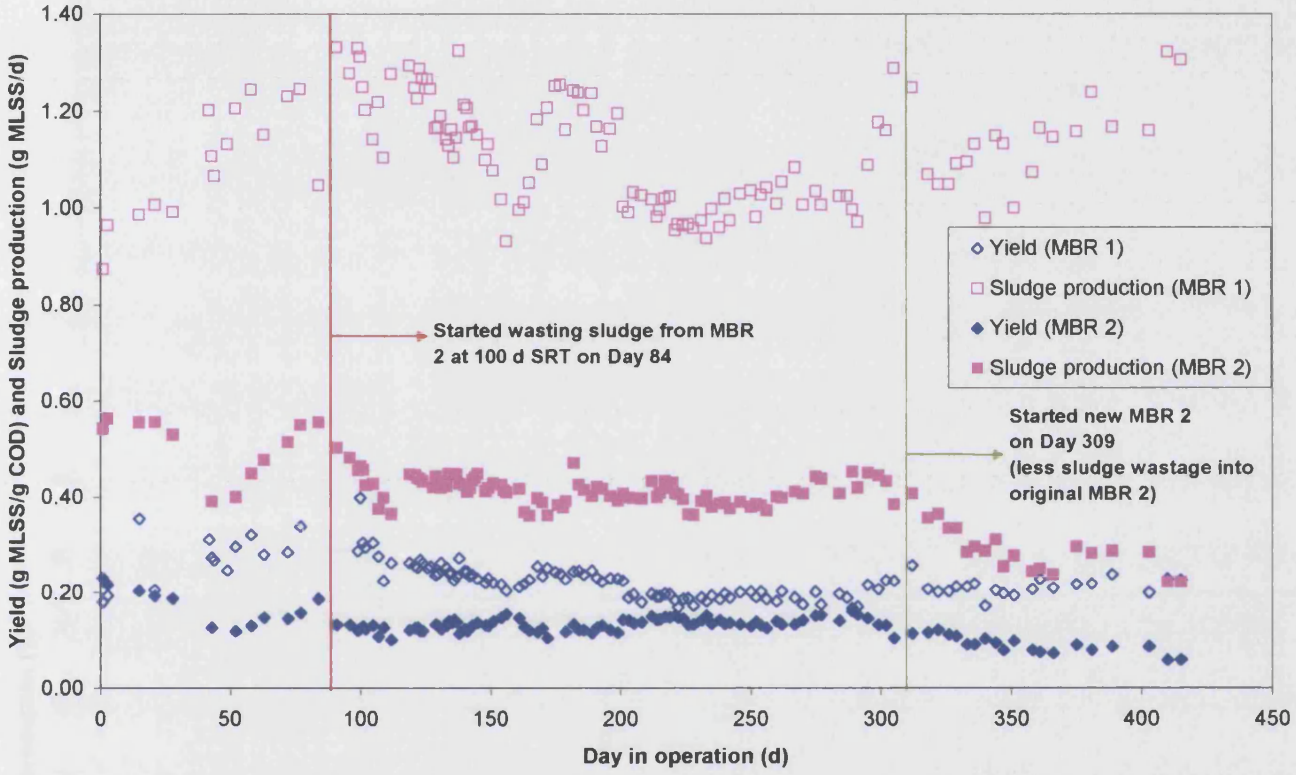


Figure 4.4: Yield and sludge production of MBRs against time (Operating conditions of MBR 1: 6 h HRT, 16 d SRT; MBR 2: 19 d HRT, 100 d SRT)

Table 4.5 shows a comparison of sludge yield of the MBRs from this work with the different treatment processes in the literature. The sludge yield of MBR 1 alone is still lower in comparison to the yield for conventional AS process, trickling filter and granular media biological aerated filter (BAF). This is expected because MBR offers the perfect control of sludge age (i.e. the system could be operated at long SRT), which in turn leads to a reduced sludge production. The comparison from Table 4.5 also shows that the two-stage MBR has the lowest sludge yield among all the

processes. This further validates the feasibility and proves the potential of the two-stage MBR concept in terms of reducing the overall sludge production.

Table 4.5: Sludge yield for various wastewater treatment processes (Mayhew and Stephenson, 1997).

Treatment Processes	Typical F/M ratio or Organic Loading rate	Typical Sludge yield (kg MLSS/ kg BOD)
Submerged MBR	0.02 – 0.04	0.0 – 0.3
Two-stage MBR (from this work)	*0.037*	*0.13 ±0.01*
MBR 1 (from this work)	*0.35*	*0.31 ±0.03*
Trickling filter	0.06 – 0.12	0.3 – 0.5
Conventional activated sludge	0.25 – 0.5	0.7 -1.0
Granular media BAF	0.5 – 1.2	0.63 – 1.06

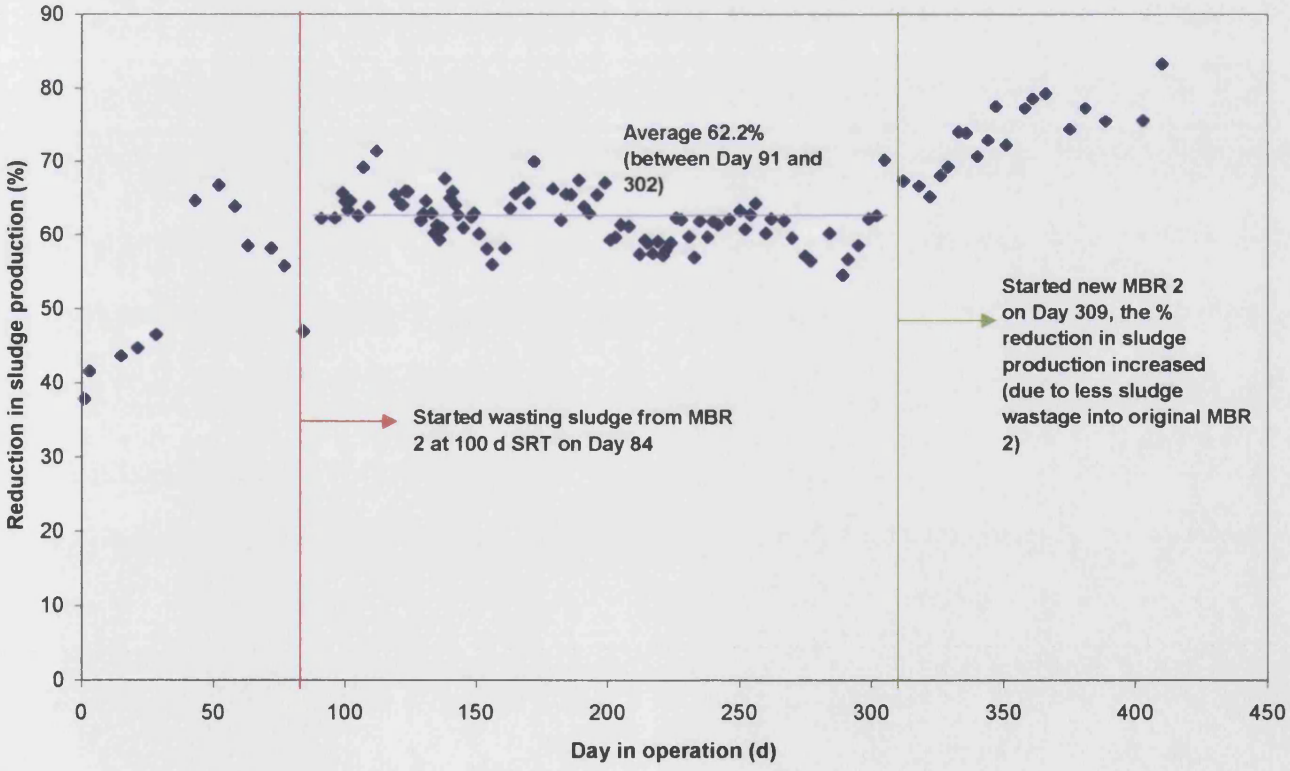


Figure 4.5: Reduction in sludge production (%) of 2-stage MBR in comparison to single MBR 1 (Operating conditions of MBR 1: 6 h HRT, 16 d SRT; MBR 2: 19 d HRT, 100 d SRT)

Figure 4.5 shows the percentage reduction in sludge production of 2-stage MBR in comparison to single MBR 1. It can be observed that the percentage reduction in sludge production increased steadily after sludge was wasted from MBR 2, from an initial value of 37.9 % on Day 1 to an average value of 62.2 ± 3.3 % between Day 91 and Day 302, before the start-up of the new MBR 2. The results clearly show that the concept of a two-stage MBR is successful in leading to a decreased excess sludge production of the system. From Day 309, the new MBR 2 was started up, hence less sludge wastage went into the original MBR 2 and this led to an increase in the percentage reduction in sludge production.

4.2.6 Comparison of volumetric sludge production and volumetric removal rate of the MBRs

4.2.6.1 Volumetric sludge production (VSP) rate

Figure 4.6 shows a comparison of the volumetric sludge production (VSP) rates of the single MBR 1 and the two-stage MBR configuration. It can be seen that the single MBR 1 has a higher VSP than the two-stage MBR configuration. From Day 121 onwards to Day 302 (before the start-up of the new MBR 2 on Day 309), the average VSP of MBR 1 was 0.31 ± 0.03 kg MLSS/m^3.d. For two-stage MBR, since the sludge was wasted at 100 d SRT on Day 84, the VSP had slowly reached a steady state from Day 121 onwards. The average VSP of the two-stage MBR from between Day 121 and Day 302 was 0.12 ± 0.007 kg MLSS/m^3.d. With the two-stage MBR configuration, the advantage of it is seen in the much lower volumetric sludge production rate. In comparison to a single stage MBR (MBR 1 only), it shows a reduction of 61.3%. This has huge economic savings on the overall sludge disposal and treatment cost, which contributes towards 25 – 65% of the total plant operation cost (Horan, 1990; Zhao and Kugel, 1997). Moreover, less volumetric sludge production also means a positive environmental impact since it was predicted that at least 50% more sludge would be produced by the end of 2005 (Mayhew, 1999) due to the necessary requirements of Directive 91/271/EEC concerning urban waste water treatment, particularly with regard to discharges to tidal waters.

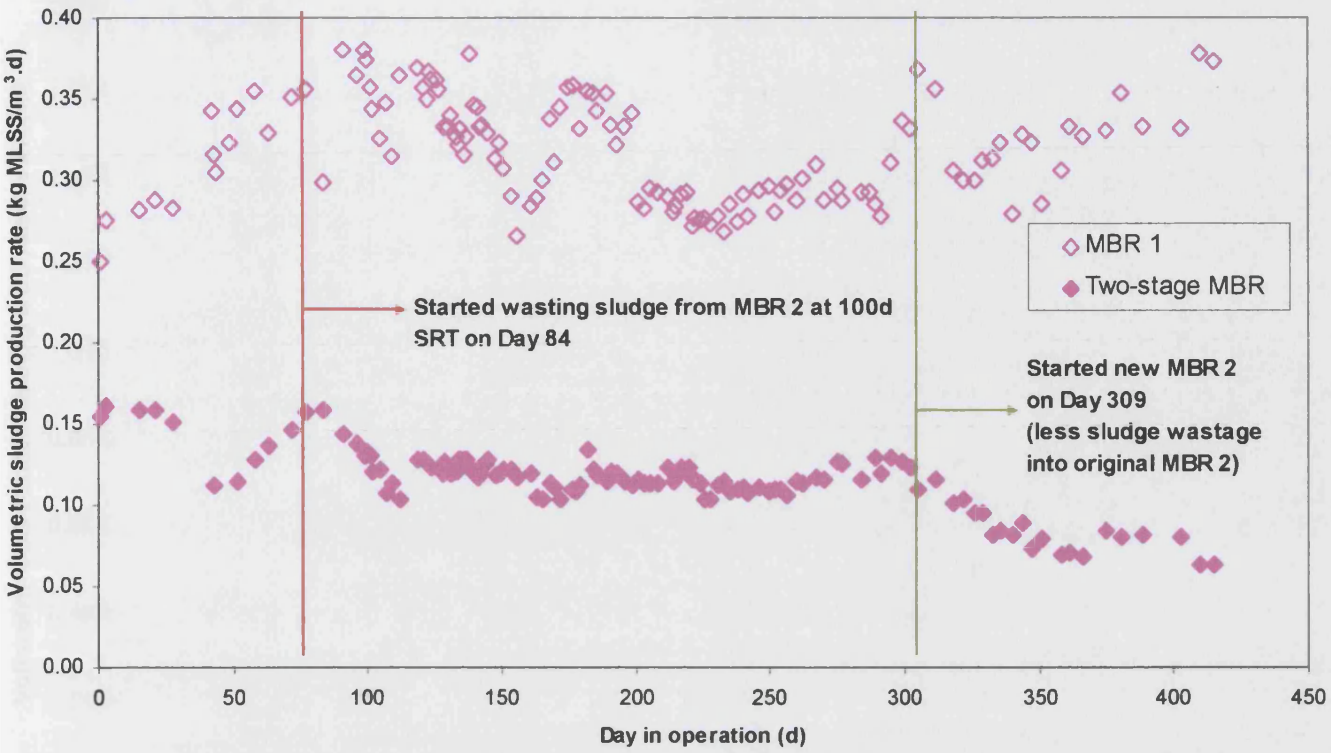


Figure 4.6: Volumetric sludge production rate of MBR1, MBR2 and 2-stage MBR against time (Operating conditions of MBR 1: 6 h HRT, 16 d SRT; MBR 2: 19 d HRT, 100 d SRT)

4.2.6.2 Volumetric COD removal rate

It can be seen from Figure 4.7 that MBR 1 had a higher efficiency in the biodegradation of organic waste compared to MBR 2. The volumetric COD removal rate of MBR 1 was overall 70 % higher than MBR 2. The noise of the graph between Day 1 and Day 77 was caused by the discrepancy in the COD value of feed measured with the old DohrMann DC-180 Carbon Analyzer. Between Day 121 and Day 302, the average removal rate of MBR 1 was 1.45 ± 0.086 kg COD removed/m^3.d whereas MBR 2 had an average value of 0.44 ± 0.042 kg COD removed/m^3.d. These results are in accordance to the proposed concept for the two-stage MBR configuration, where the first stage is focused on high COD / BOD removal rate and the second stage is focused on reduced sludge production rate.

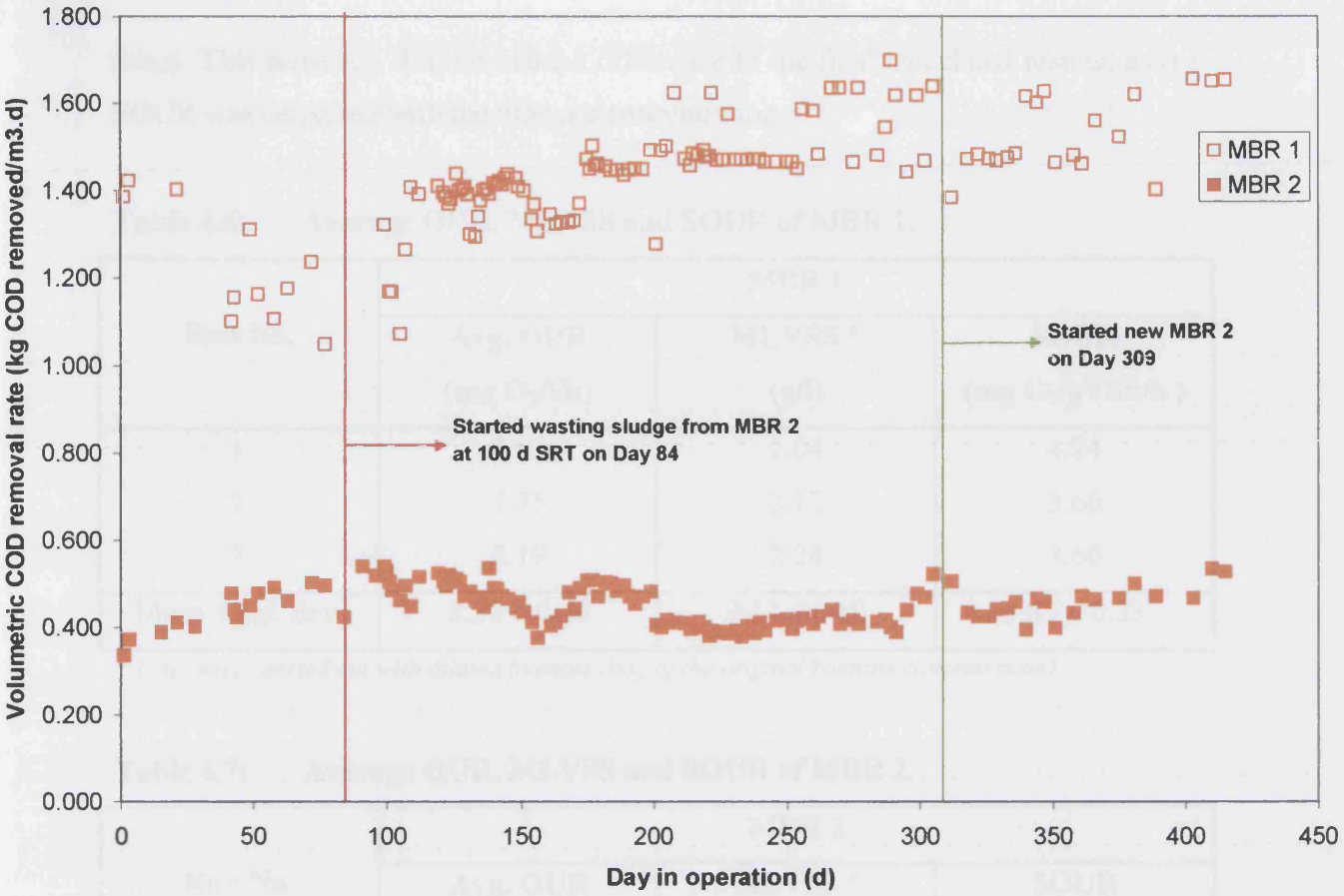


Figure 4.7: Volumetric COD removal rate of MBR 1 and MBR 2 against time (Operating conditions of MBR 1: 6 h HRT, 16 d SRT; MBR 2: 19 d HRT, 100 d SRT)

4.3 Specific Oxygen Uptake Rate (SOUR)

The combination of the oxygen uptake rate (OUR) with the concentration of MLVSS yields a value termed "specific oxygen uptake rate" (SOUR), or respiration rate. SOUR is an indication of the amount of oxygen used by microorganisms (the biological activity of the microorganisms in the bioreactor) and is defined as oxygen uptake rate per unit of MLVSS per time, i.e. g O_2 /g MLVSS/h (Metcalf and Eddy, 1991). Tests with the OxiTop®, WTW respirometer were carried out to find the SOUR of the two MBRs and the results are listed in Table 4.6 and Table 4.7. Tests were carried out with diluted biomass (with diluent water) taken from the MBRs, as the undiluted biomass concentration was too concentrated for respirometry and the oxygen uptake rate measured by the OxiTop® was out of the specified measurement

range. This however, did not make a difference to the final calculated results, as the SOUR was corrected with the biomass concentration.

Table 4.6: Average OUR, MLVSS and SOUR of MBR 1.

Run No.	MBR 1		
	Avg. OUR ($mg O_2/l/h$)	MLVSS * (g/l)	SOUR ($mg O_2/gVSS/h$)
1	8.67	2.04	4.24
2	7.75	2.12	3.66
3	8.19	2.24	3.66
Mean ± std. dev.	8.20 ± 0.46	2.13 ± 0.10	3.85 ± 0.33

** Tests were carried out with diluted biomass (half of the original biomass concentration)*

Table 4.7: Average OUR, MLVSS and SOUR of MBR 2.

Run No.	MBR 2		
	Avg. OUR ($mg O_2/l/h$)	MLVSS * (g/l)	SOUR ($mg O_2/gVSS/h$)
1	5.56	5.28	1.05
2	5.78	6.13	0.94
3	3.36	4.18	0.81
4	3.58	3.29	1.09
5	5.86	3.29	1.78
Mean ± std. dev.	4.83 ± 1.25	4.43 ± 1.25	1.13 ± 0.38

** Tests were carried out with diluted biomass (half of the original biomass concentration)*

It can be seen from Table 4.6 and Table 4.7 that there was a significant difference in the SOUR of MBR 1 and MBR 2. MBR 1 has a higher average SOUR value of 3.85 ± 0.33 mg O_2/gVSS/h whereas MBR 2 has an average SOUR value of 1.13 ± 0.38 mg O_2/gVSS/h. This indicates that MBR 1 has a higher biological activity than MBR 2. This is due to the biomass being more viable and active in metabolising the synthetic feed in MBR 1; while MBR 2 is more focused on cell lysis and endogenous respiration. The SOUR results correspond well with the concept that was proposed for the two-stage MBR system.

4.4 New MBR 2

Due to the algae bloom in the original MBR 2 at the start up, a new 2nd stage (new MBR 2) was started. This was aimed at investigating the trend of development of the original 2nd stage MBR without algal bloom, and these include the pH changes, the microscopic examination of the higher organisms found, the development of the biomass and the permeate COD change. The new MBR 2 was of a smaller scale than the original MBR 2 (1 L compared to 3.5 L) in order that the system could reach steady state in a shorter period of time. A Millipore crossflow membrane module was used with a 0.45 μm polymeric Millipore membrane. The new MBR 2 was immersed into a water bath kept at a constant temperature of 20 – 22 °C. The schematic of the system and a photograph of the apparatus can be referred to in Chapter 3.

The new MBR 2 was started up on Day 309 and it took about a week to fill up to 1 L. At the start, the sludge wasted from MBR 1 into the original MBR 2 was stopped and went into the new MBR 2. But the sludge wastage from MBR 1 was halved when the sludge concentration was quite high and half of it went into the original MBR 2.

4.4.1 pH changes of the new MBR 2

Figure 4.8 shows the pH changes recorded for the new MBR 2 since start up on Day 309 until Day 441 for 132 days. It can be seen that there was a gradual drop since start up on Day 309 from a pH of 6.6 to 4.6 on Day 441. This was due to the nitrification activity as confirmed with the nitrification test as shown later in Section 4.5. As mentioned in Section 4.2.4.2, the original MBR 2 also went through a gradual pH drop since the start up. However, the original MBR 2 suffered from a much more severe acidity shock as the pH dropped as low as 3.8 on Day 240. The explanation for the pH drop is given in Section 4.5.4.

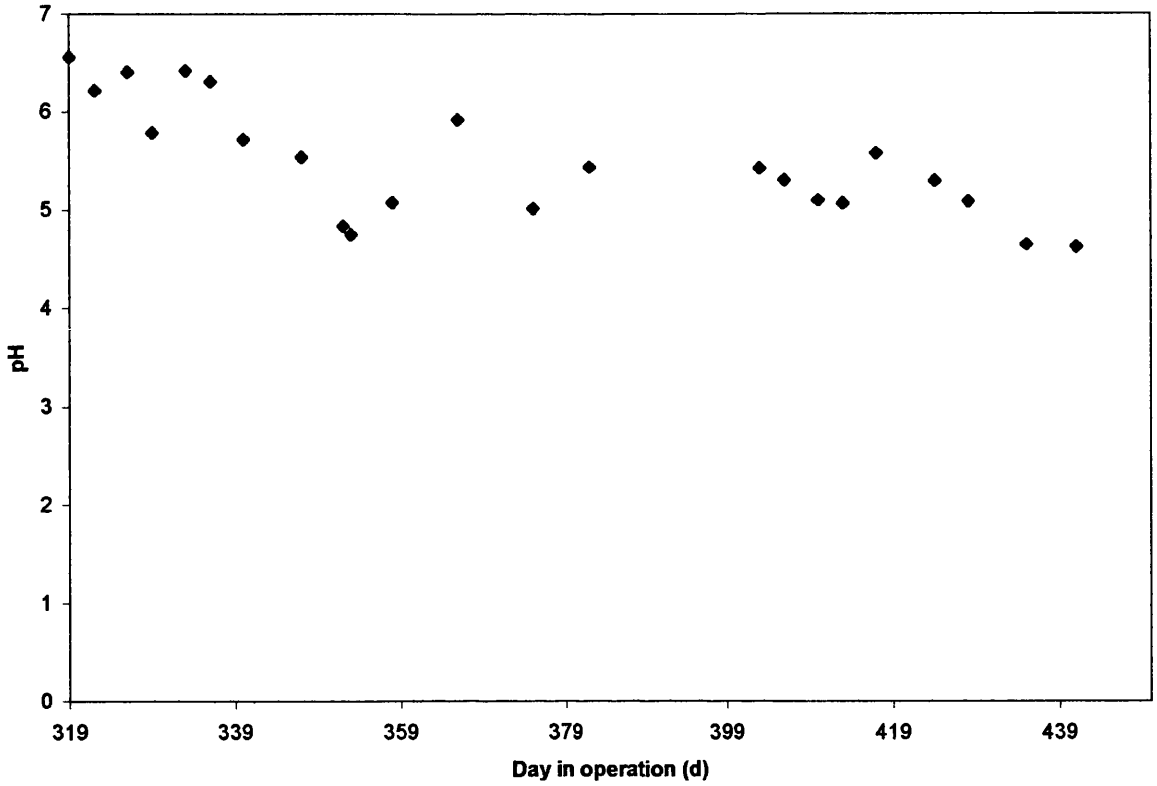


Figure 4.8: pH changes of the new MBR 2 against time

4.4.2 Development of biomass and permeate COD

As seen from Figure 4.9, the biomass concentration increased rapidly upon start up from 7,900 mg/l and 7,300 mg/l (for MLSS and MLVSS respectively) on Day 319 to 18,200 mg/l and 16,400 mg/l on Day 336. The sludge was so thick that water evaporated easily with the high air flow rate (0.02 l of air/l of culture volume/min) and the high temperature during the summer months; hence some distilled water was added to the system. The air flow was also turned down to 0.016 l of air/l of culture volume/min but the dissolved oxygen concentration was always maintained at above 2 mg/l. The sludge input wasted from MBR 1 into the new MBR 2 was also halved and the remainder went back into the original MBR 2. It could be observed that the biomass concentration oscillates to what seems to be steady state values of 7,000 mg/l and 6,500 mg/l (for MLSS and MLVSS respectively). This trend was disturbed as a pump failure caused the system to be halted on Day 390 and most of the biomass in the new MBR 2 was lost. The system was started up again on Day 391 and the biomass was seen increasing gradually to maximum MLSS and MLVSS values of 9,900 mg/l and 9,000 mg/l on Day 428. This then saw a drop followed by an increase

to 8,800 mg/l and 8,000 mg/l respectively on Day 441. The average MLVSS/MLSS ratio was very high at 91 %, showing the sludge was mainly organic solids and little inorganic solids was present in the new MBR 2.

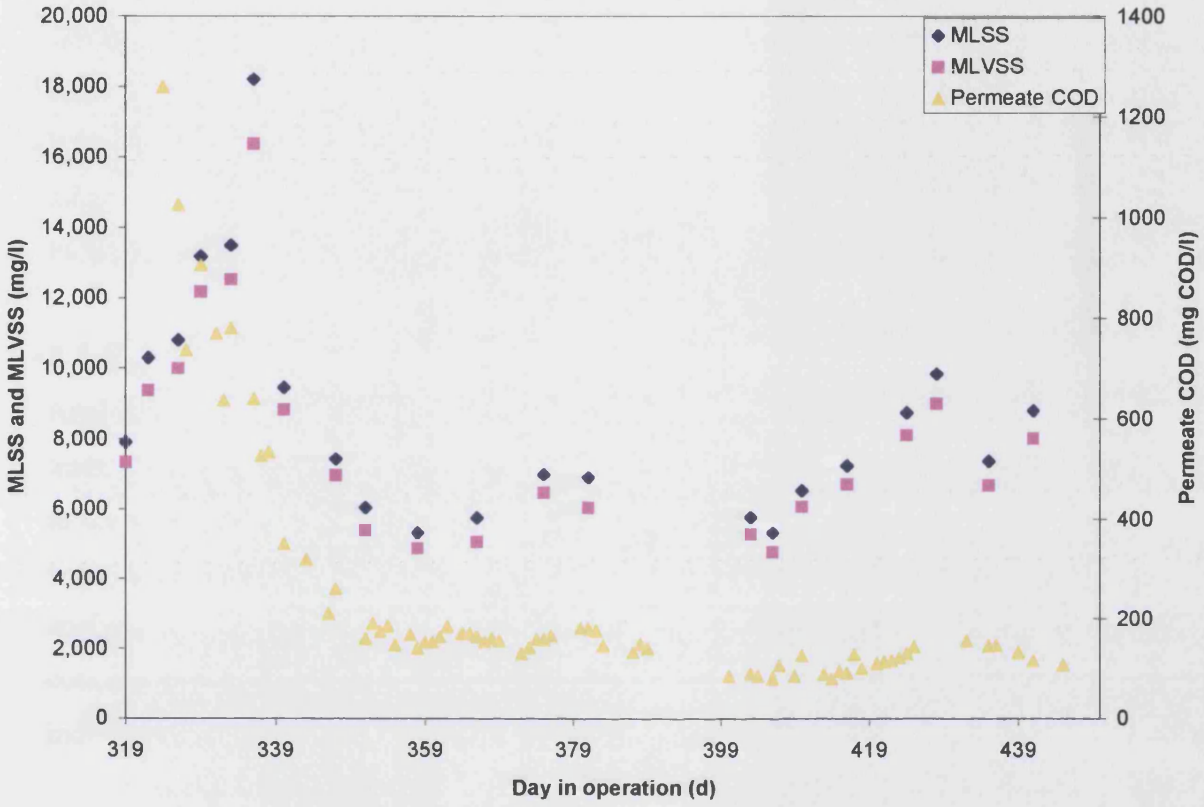


Figure 4.9: Change in permeate COD and development of MLSS and MLVSS of the new MBR 2 against time (Operating condition of new MBR 2: 9.3 d HRT, 100 d SRT)

Figure 4.9 also shows the profile of permeate COD in the new MBR 2. The permeate COD started off with a very high value of 1,260 mg COD/l on Day 324. This initially showed an exponential decrease for the first four months it was in operation. It can be seen that the permeate COD levelled off starting Day 358, around a value of 140 mg COD/l. This was brought to a halt when a pump failure occurred on Day 390 and the system had to be restarted. The permeate COD was therefore much lower as seen from Day 400 to Day 414 due to a lower biomass. As the biomass gradually built up, the permeate COD also increased gradually. However, the permeate COD then decreased and reached around 100 mg COD/l on Day 418. The permeate COD is seen to be affected slightly by the development of the biomass at the second start up after

the pump failure. This was most probably due to a higher amount of lysate being released from cell lysis at a higher biomass concentration.

4.4.3 Observation and microscopic examination of the sludge

The sludge in the new MBR 2 was similar to MBR 1, i.e. it was a yellowish, orange colour. Microscopic examination showed the presence of higher organisms at the start. These included ciliated protozoa, rotifers and nematodes. Even when the system was turning acidic with time, a healthy population of protozoa that had apparently adapted and survived under their new environment could still be observed. The microscopic pictures of the different higher organisms can be seen in Chapter 6.

4.5 Nitrogen concentration in the MBRs

Analysis of nitrogen concentration of the MBRs was carried out with the Hach TNT tests, (Hach, USA). Nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_3) concentrations in the synthetic feed stream and permeate streams of MBR 1, MBR 2 and new MBR 2 were tested and the results are shown in Figure 4.10 – 4.13. As the nitrogen test analysis is very costly, only single measurement was carried out for each nitrogen concentration at any time. However, the average values were calculated from the individual tests on different days of analysis and are summarised in Table 4.8.

Table 4.8: Average values of the nitrogen concentrations in permeate of MBR 1 (P1), original MBR 2 (P2), new MBR 2 (nP2) and synthetic feed.

	Nitrate (mg NO_3^-/l)	Nitrite (mg NO_2^-/l)	Ammonia (mg NH_3/l)
Feed	0.23	0.0075	2.50
P1	3.24	14.11	27.50
P2	74.50	0.0056	60.38
nP2	163.6	1.05	166.6

4.5.1 Synthetic Feed

Figure 4.10 shows the nitrogen concentration in the synthetic feed stream. The nitrate and nitrite concentrations are very low, as the nitrogen was mostly organic and in the form of meat extract, peptone and urea. According to the COD:BOD:TN:Ammonia:P = 140:65:11:7:1.5 – 2 ratio for the synthetic feed (see Chapter 3), the total nitrogen concentration (TN) corresponding to a feed COD of 440 mg COD/l will be 35 mg N/l, while the corresponding ammonia concentration will be 22 mg NH_3/l. However, the ammonia concentration measured was very low, varying from 2 – 4 mg NH_3/l. The fluctuation is more likely due to the variation in the feed ammonia concentrations during the preparation of the synthetic feed at each different batch. The total nitrogen (TN) of the synthetic was not able to be verified and hence the accuracy of the standardized ratio was not confirmed. Nevertheless, the nitrogen balance in the later section proved the accuracy of the measured nitrogen results reported here. Table 4.9 shows the typical nitrogen concentration found in untreated domestic wastewater (Metcalf and Eddy, 1991).

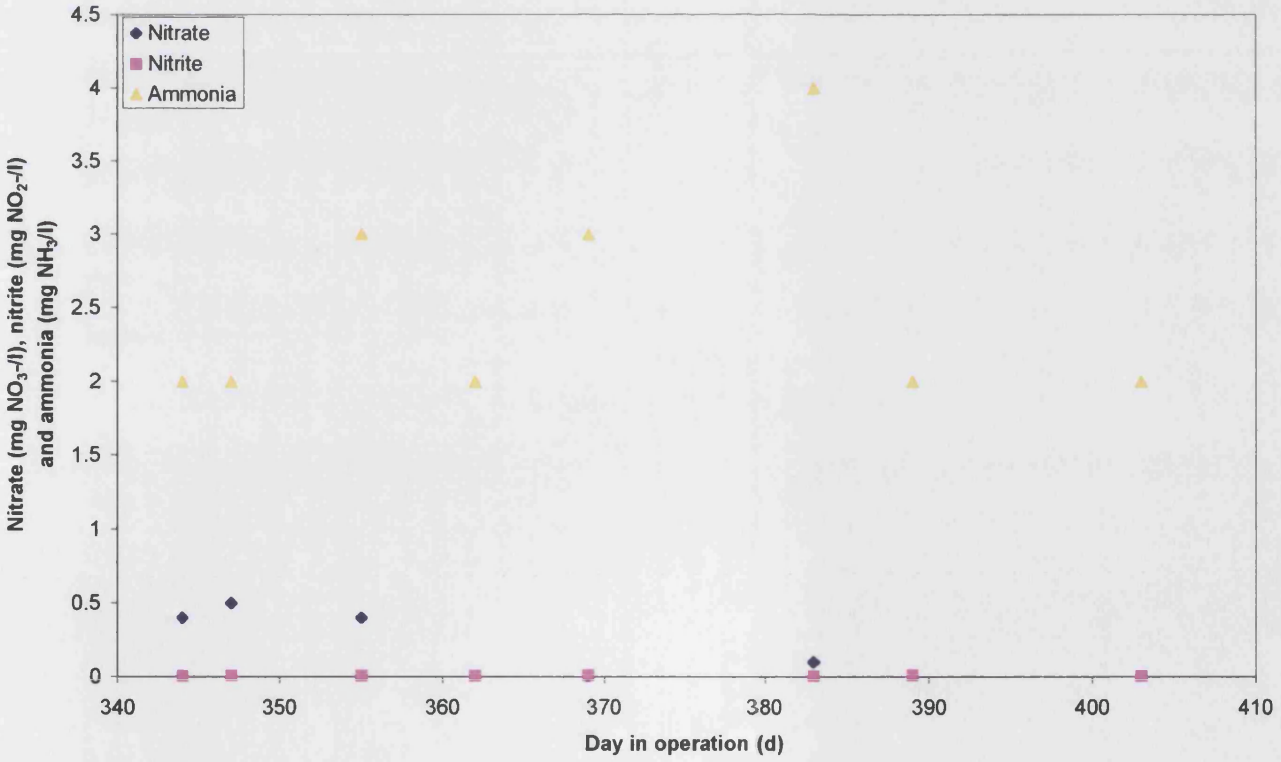


Figure 4.10: NO_3^-, NO_2^- and NH_3 concentrations in the synthetic feed

Table 4.9: Typical nitrogen concentration of untreated domestic wastewater (Metcalf and Eddy, 1991).

Contaminants (mg/l)	Concentration		
	Weak	Medium	Strong
Nitrogen (total as N)	20	40	85
Organic	8	15	35
Free ammonia	12	25	50
Nitrites	0	0	0
Nitrates	0	0	0

4.5.2 Permeate of MBR 1

Figure 4.11 shows the nitrogen concentrations in the permeate of MBR 1. It can be seen that the ammonia and nitrite concentrations are very high in the permeate. The nitrate concentration, on the other hand, was much lower in comparison. The high ammonia concentration (average value of 27.50 mg NH_3/l) may be attributed to bacteria breaking down the organic nitrogen matter (meat extract, peptone and urea) in the synthetic feed. Also, the bacterial cell lysis releases ammonia nitrogen to the system. During the mineralization process by protozoal grazing, ammonium and phosphate are also common mineralization products. Lee and Welander (1996) reported a significant release of nitrate (7-13 mg N/l) and of phosphate (2.5-3.7 mg P/l) in the predator stage using protozoa and metazoa due to the mineralization activities of the predators (i.e. the oxidation of bacteria).

The presence of nitrate and nitrite ions in the permeate of MBR 1 shows that there was nitrification in the system. But the higher nitrite concentration (average value of 14.11 mg NO_2^-/l) compared to nitrate concentration (average value of 3.24 mg NO_3^-/l) shows that there was some kind of inhibition to the nitrification process. The nitrification process comprises two sets of reactions involving two bacterial species of nitrifiers (*Nitrosomonas* sp. and *Nitrobacter* sp.) and is described by Equations 4.1 and 4.2 (Metcalf and Eddy, 1991):

Nitrosomonas sp. oxidize ammonia (NH_3) to nitrite (NO_2^-): (4.1)

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \longrightarrow C_5H_7O_2N + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3$$

Nitrobacter sp. convert nitrite (NO_2^-) to nitrate (NO_3^-): (4.2)

$$400\ NO_2^- + NH_4^+ + 4\ H_2CO_3 + HCO_3^- + 195\ O_2 \longrightarrow C_5H_7O_2N + 3\ H_2O + 400\ NO_3^-$$

In Equation 4.1, ammonia (NH_4^+) is combined with oxygen (O_2) and hydrogen carbonate (HCO_3^-) to produce the intermediate product, nitrite (NO_2^-), bacterial cell mass, water and carbonic acid. In Equation 4.2, nitrite is combined with ammonia, carbonic acid, hydrogen carbonate and oxygen to produce bacterial cell mass, water and lots of nitrate (NO_3^-). The conversion from ammonia to nitrite involves a complex series of reactions that control the overall conversion process as evidenced by the lack of nitrite build-up in the system (Metcalf and Eddy, 1991). Therefore, the conversion of ammonia to nitrite is the rate-limiting step and hence the nitrate concentration will always be higher than the intermediate nitrite concentration. However, as observed from Figure 4.11, there was a build up of intermediate nitrite and hence it was deduced that the nitrification process was inhibited.

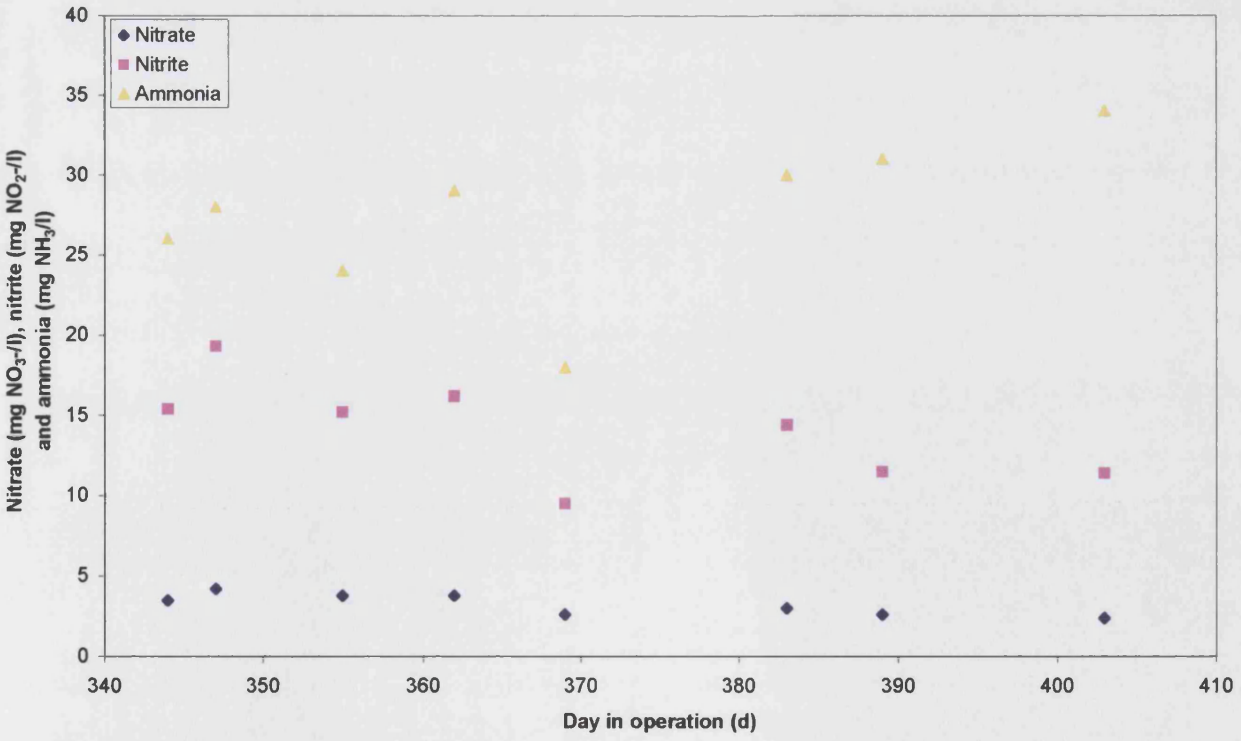


Figure 4.11: NO_3^-, NO_2^- and NH_3 concentrations in permeate of MBR 1 (Operating conditions of MBR 1: 6 h HRT, 16 d SRT)

Nitrifying bacteria are sensitive organisms and extremely susceptible to a wide variety of inhibitors (Metcalf and Eddy, 1991). A variety of organic and inorganic agents can inhibit the growth and action of these organisms. For instance, high concentrations of ammonia and nitrous acid can be inhibitory. The effect of pH, temperature and dissolved oxygen concentration can also be significant. It was not possible to identify what type of inhibition or which inhibitory agent was taking place in MBR 1, which resulted to the build-up of nitrite concentration. Nevertheless, the measured nitrogen results show nitrification occurring in both of the MBRs.

4.5.3 Permeate of original MBR 2

Figure 4.12 shows the nitrogen concentrations in the permeate of the original MBR 2. It can be observed that the ammonia and nitrate concentrations were very high in the permeate, while nitrite concentration was very low.

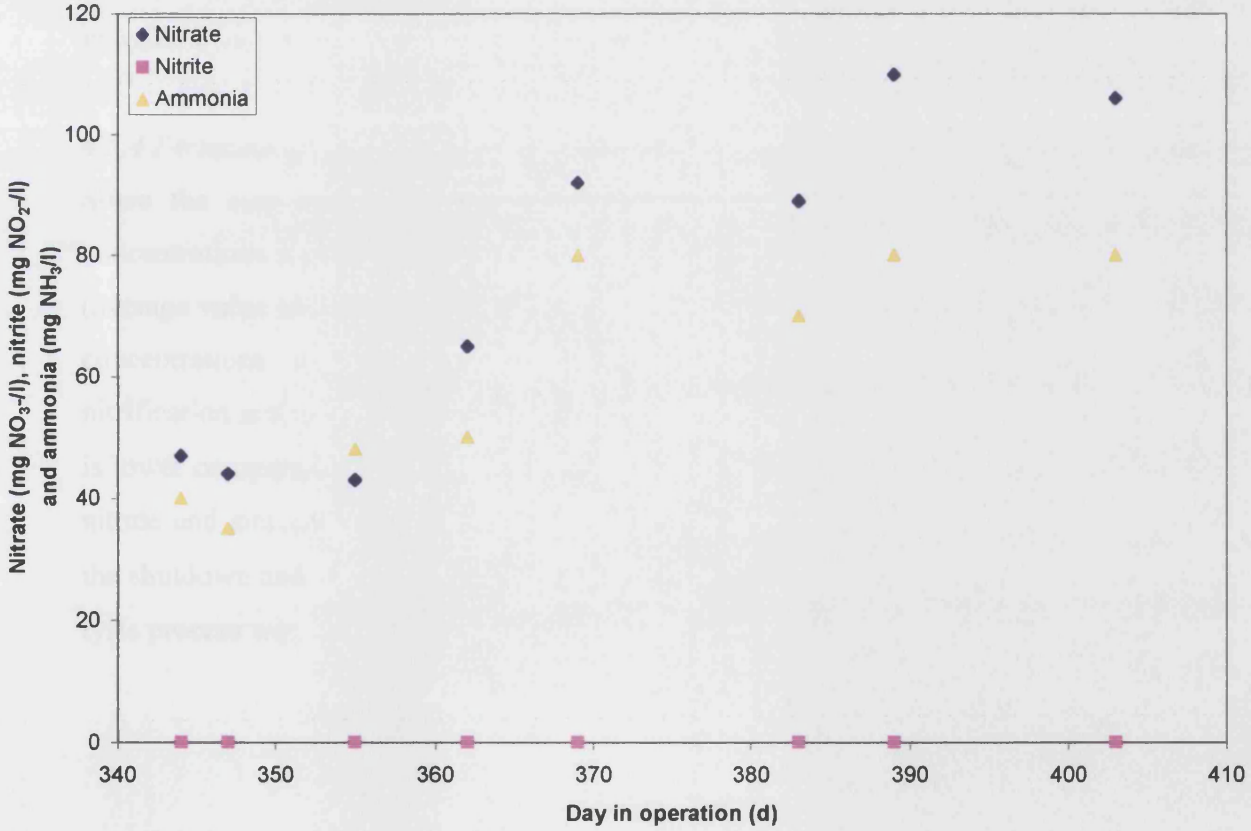


Figure 4.12: NO_3^-, NO_2^- and NH_3 concentrations in permeate of original MBR 2 (Operating conditions of original MBR 2: 19 d HRT, 100d SRT)

Also, there was an increasing trend in the ammonia and nitrate concentration since Day 355. This can be explained by the restart of sludge wastage from MBR 1 into MBR 2. Before that, the sludge from MBR 1 was wasted into the new MBR 2 upon its startup on Day 309. However, when the sludge concentration was increasing in the new MBR 2, the sludge wastage was halved and the other half was wasted into the original MBR 2. The ammonia and nitrate concentrations seemed to have reached a steady state in starting Day 389.

The average values of nitrate, nitrite and ammonia were 74.50 mg NO_3^-/l, 0.0056 mg NO_2^-/l and 60.38 mg NH_3/l respectively. Both nitrate and ammonia concentrations in the permeate of MBR 2 are higher compared to that in the permeate of MBR 1. This may be explained by the focus of the MBR 2 in cell lysis, where more cells are broken down into organic nitrogen matter, which contributed to the much higher ammonia and nitrate concentrations. As seen from Figure 4.12, there was no build up of nitrite in MBR 2 like in MBR 1.

4.5.4 Permeate of the new MBR 2

Since the start-up of the new MBR 2 on Day 309, the nitrate and ammonia concentrations were increasing gradually as the biomass was building up. The nitrate (average value of 163.6 mg NO3-/l) and ammonia (average value of 166.6 mg NH3/l) concentrations were the highest among all the MBRs. There was significant nitrification activity occurring in the new MBR 2. However, the nitrite concentration is lower compared to that of MBR 1 (average value of 14.11 mg NO2-/l). The lower nitrate and ammonia concentrations on Day 403, as seen in Figure 4.13 were due to the shutdown and restart-up of the new MBR 2 on Day 391. This was because the cell lysis process was not as prominent in the MBR during the start up.

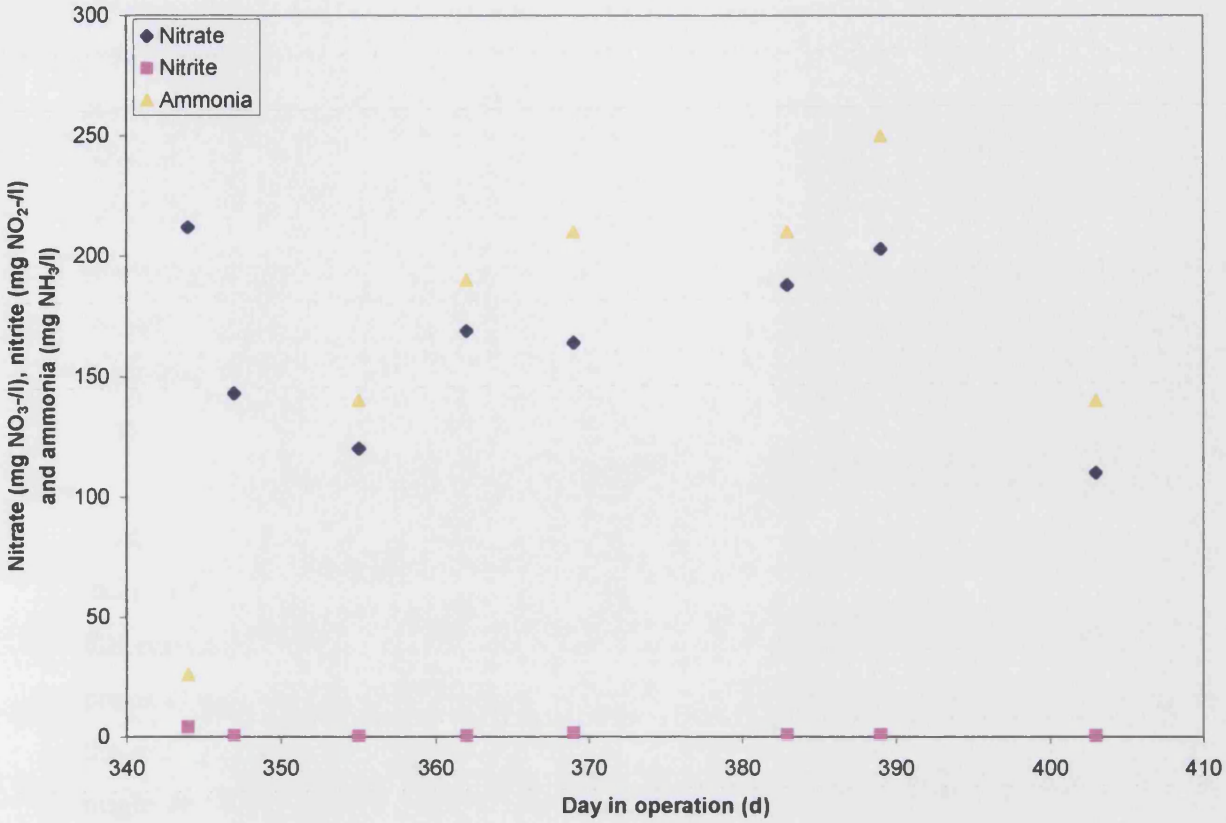


Figure 4.13: NO_3^-, NO_2^- and NH_3 concentrations in permeate of new MBR 2 (Operating conditions of new MBR 2: 9.3 d HRT, 100d SRT)

In the literature, concerns are reported about the possible severe effects of protozoan grazing on slow-growing nitrifiers than for fast-growing heterotrophs. Due to intensive grazing, the nitrifier population could reduce to a level that can no longer be capable of bringing about nitrification. Bouchez *et al.* (1998) observed a complete breakdown of nitrification due to over-growth of protozoa in a sequencing batch reactor. Lee and Welander (1994) observed a disturbance of nitrification activity in an aerobic biofilm system. However, others did not have the same observation in an activated sludge system (Rensink and Rulkens, 1997). In fact, it was found that the presence of flagellates could enhance the nitrification activity in soils (Verhagen and Laanbroek, 1992; Verhagen *et al.*, 1993). Verhagen and Laanbroek (1992) found that the rate of nitrate production (an indicator of nitrification activity) is the same with or without the presence of protozoa. Results even indicate a higher activity per nitrifying cell in the presence of protozoa/flagellates. This could be explained by the production

of ammonium, NH_4^+ through the mineralisation process in the presence of protozoa, which enhances the nitrification activity. It was found that during the protozoan grazing, one-third of the nitrogen of digested bacteria was converted into protozoan biomass; one-third consisting mainly of cell walls and organelles, was excreted as indigestible organic nitrogen; and the other one-third was excreted as ammonium (Goldman *et al.*, 1985). Therefore, protozoan grazing may stimulate the growth of N-limited heterotrophic bacteria and plants (Elliot *et al.*, 1979; Clarholm, 1985; Kuikman and van Veen, 1989) or may increase the nitrification rate (Griffiths, 1989) with the enhanced turnover rate of nitrogen.

Clearly, in the case for MBR 1, MBR 2 and new MBR 2, there was nitrification as indicated by the nitrate concentrations. However, there was some kind of inhibition on the conversion of nitrite to nitrate in MBR 1. On the other hand, the nitrification process was not efficient enough to support the ammonia nitrogen load produced from the cell lysis in both MBR 2 and new MBR 2. It was concluded a new process design might be able to solve the problem of high nitrite concentration in MBR 1 and high ammonia and nitrate concentrations in the 2nd stage. A higher sludge retention time (SRT) in the MBRs will increase the sludge concentration and ensure that there are sufficient nitrifiers in the system to cope with the high ammonia nitrogen load, especially those produced from the cell lysis in both MBR 2 and new MBR 2. Apart from that, a higher temperature will also increase the nitrification rate of the nitrifiers. In the large-scale wastewater treatment plant (such as Swanage), it is operated with an anoxic tank before the MBR acting as the denitrification tank. Return of activated sludge to the denitrification tank is flow balanced to ensure adequate removal of nitrate (Churchouse, 1997).

During the nitrification process, approximately 4.3 mg O_2 are consumed for every mg of ammonia-nitrogen (NH_4^+) oxidised to nitrate-nitrogen (NO_3^-). Also, 8.64 mg of alkalinity in the form of HCO_3^- are consumed per mg of ammonia-nitrogen (NH_4^+) oxidized (Metcalf and Eddy, 1991). This is quite a substantial amount of alkalinity and will over a period of time dramatically change the character of the system, affecting both hardness and pH stability. It is also an acidifying process, producing a gradual build up of nitric acid. Hence, the original MBR 2 and the new MBR 2 had

seen a gradual pH drop over the period where they had been in operation. MBR 1 however, did not experience such a dramatic pH change.

4.5.5 Nitrogen balance around the MBR

Mass balance analysis was carried out for the nitrogen around the MBRs to confirm the results obtained from the nitrogen measurements. Mass balance equations for MBR 1 and MBR 2 may be written as Equations 4.3 and 4.4:

MBR 1:

Mass flow of N into MBR 1 (in synthetic feed) = Mass flow of N out of MBR 1 (in permeate + in biomass being wasted) (4.3)

MBR 2:

Mass flow of N into MBR 2 (in permeate + in biomass wasted from MBR 1) = Mass flow of N out of MBR 2 (in permeate + in biomass being wasted) (4.4)

According to the COD:BOD:TN:Ammonia:P = 140:65:11:7:1.5 – 2 ratio for the synthetic feed, the total nitrogen concentration (TN) corresponding to a feed COD of 440 mg COD/l will be 34.7 mg N/l. As for the biomass, the following stoichiometry was assumed: $C_5H_7O_2N$ (Metcalf and Eddy, 1991). The total molecular weight for the biomass is 113 while for nitrogen is 14.007. Therefore, the mass fraction of N in biomass is 0.124.

Table 4.7: MLVSS and inlet, permeate and sludge flowrates for MBR 1 and MBR 2.

	MBR 1	MBR 2
Biomass MLVSS (g/l)	5.02	6.77
Inlet Flowrate, Q_0 (l/h)	0.583	0.0091
Permeate Flowrate, Q_P (l/h)	0.574	0.0077
Sludge wastage, Q_w (l/h)	0.0091	0.0015

For MBR 1:

Mass flow of N in Feed = 20.24 mg N/h (calculated from TN ratio in feed)

Mass flow of N in permeate of MBR 1 = 15.43 mg N/h (measured N)

Mass flow of N in biomass wasted from MBR 1 = 5.67 mg N/h (calculated from stoichiometry)

N into MBR 1 = N out of MBR 1

20.24 mg N/h = 21.10 mg N/h

Discrepancy = 0.86 mg N/h

The above nitrogen balance analysis for MBR 1 showed a minor discrepancy but shows that the nitrogen concentrations measured by the test kits were reasonable.

For MBR 2:

Mass flow of N in permeate of MBR 1 = 0.2721 mg N/h (measured N)

Mass flow of N in biomass wasted from MBR 1 = 0.0057 mg N/h (calculated from stoichiometry)

Mass flow of N in permeate of MBR 2 = 0.2965 mg N/h (measured N)

Mass flow of N in biomass wasted from MBR 2 = 0.0012 mg N/h (calculated from stoichiometry)

N into MBR 2 = N out of MBR 2

0.2778 mg N/h = 0.2977 mg N/h

Discrepancy = 0.02 mg N/h

The above nitrogen balance analysis for MBR 2 also shows a small discrepancy but shows that the nitrogen concentrations measured by the test kits were reasonable.

4.6 Membrane fouling characteristics of MBR 1 and MBR 2

4.6.1 Introduction

For pressure-driven membrane processes, fouling remains the main limiting factor of the performance and efficiency of the system. Fouling leads to flux decline and changing retention characteristics, which limits membrane performance, reduces the working life of membranes and increases the cleaning cost. Fouling behaviour could be dependent on many factors, among which are the material properties of the membrane and the nature of the foulants (Wiesner and Chellam, 1999). In the case of MBRs, membrane fouling is a result of interaction between the membrane materials and activated sludge broth. Activated sludge broth is a diverse and dynamic microbial community, which consists of suspended solids, colloids, solutes, bacteria and dissolved or macromolecular organic matter (Fan *et al.*, 2000). Hence, membrane fouling in a multi-component living system is complex. The effects of physiological states of the living system on membrane fouling might play a significant role apart from the physico-chemical parameters.

4.6.2 Theory of Critical Flux

Field *et al.* (1995) proposed the critical flux concept for membrane filtration, whereby on start-up there exists a flux below which a decline of flux with time does not occur. If this critical flux is exceeded, fouling is observed. Therefore, operating membrane filtration at a permeate flux lower or near the critical flux could reduce or eliminate irreversible membrane fouling. Knowledge of the microbial suspension's filterability and critical flux for the MBR systems could be crucial in minimizing the operation and investment cost of the membranes, which contributes to a major percentage of the process capital cost. Critical flux concept could therefore be applied to reduce membrane fouling since maintaining critical flux in membrane system is an alternative method for preventing flux reduction. The critical flux concept has previously been employed by Kubota on their submerged membrane bioreactors. Ishida *et al.* (1993) reported that under normal operating conditions flux is maintained at 0.5 $m^3.m^{-2}$ per day (or 20.83 l. m^{-2}. h^{-1} [LMH]) with steady pressure of about 30 kPa.

Critical flux can be evaluated by conducting constant flux filtration experiments, and measuring the resulting transmembrane pressure (TMP). Constant flux operation is

preferred over constant TMP filtration because the interpretation of the data from constant pressure experiments often causes problems since the flux variation produces changing conditions (of concentration, rheology, solubilities, etc.) in the boundary layer throughout any experiment (Aimar and Howell, 1989). During a typical experiment, the permeate flux is increased stepwise, keeping the crossflow velocity constant and the resulting transmembrane pressure (TMP) measured. At fluxes equal to or lower than the critical flux, the relationship between TMP and flux should be linear with no hysterisis (Wu *et al.*, 1999). For low fluxes the TMP increased linearly with flux (no fouling, sub-critical flux). Above critical flux, the TMP-versus-flux curve starts to deviate from a straight line. Wu *et al.* (1999) proposed two forms of critical flux. The "strong" form of critical flux exists if the flux of a suspension is identical to the flux of clean water at the same TMP (see Figure 4.14a). The "weak" form of critical flux exists if the relationship between flux and TMP is linear, but the slope of the line differs from that for clean water (see Figure 4.14b).

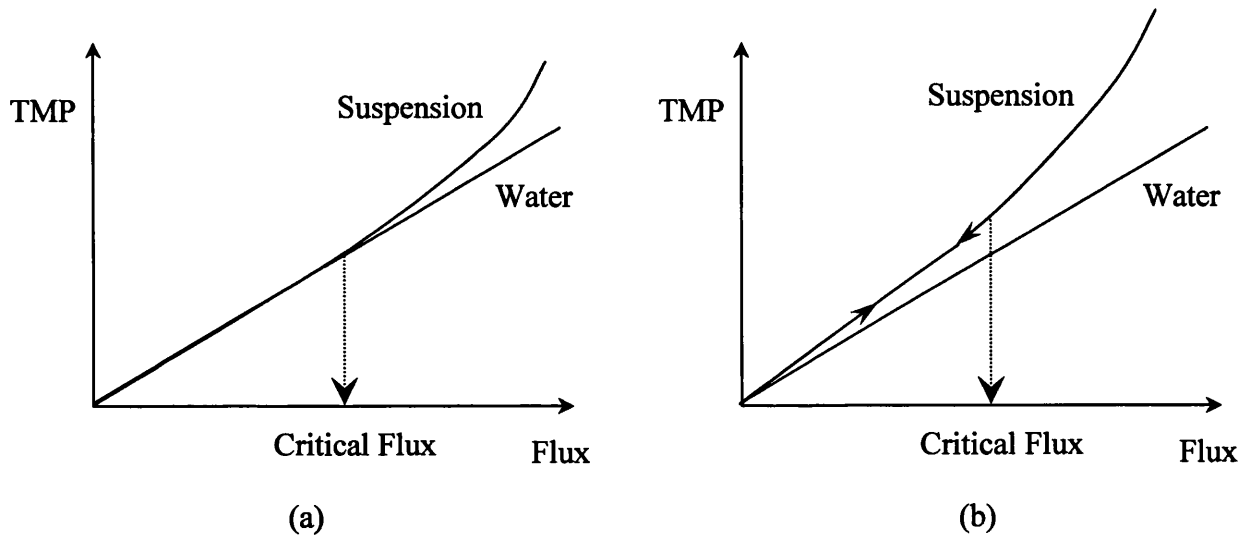


Figure 4.14: Comparison of strong form and weak form of the critical flux. (a) Strong form: up to critical flux, flux identical to clean water flux. (b) Weak form: up to critical flux, flux proportional to TMP with no hysterisis observed in flux vs TMP curve with any increases or decreases in flux (Fradin and Field, 1999).

4.6.3 Materials and Methods

The membrane was soaked in 1 % of sodium hypochlorite overnight to ensure that it was clean, and checked for pure water flux before each test run. The percentage flux recovery gives an indication if the membrane is cleaned thoroughly. A gear pump was calibrated and a balance was used to monitor the permeate flux. During a test run, the TMP was recorded using a Druck pressure transducer interfaced to a computer with GenieDac data acquisition card. The MLSS and MLVSS concentration in the MBRs were determined according to the standard methods (refer to Chapter 3). The tests were started at a low flux of 4 l. m^{-2}. h^{-1} (LMH) while TMP was measured for a period of 10 minutes. The flux was then increased at a step interval of 2 LMH until critical flux could be identified, and then flux was decreased at the same step interval. This procedure indicates whether fouling exhibits hysteresis effects or whether the TMP flux curves were completely reversible. The TMP flux curves were then compared with the pure water TMP at the same fluxes.

4.6.4 Fouling test results

The membrane was thoroughly clean as indicated by the 100 % pure water flux recovery. Figure 4.15 and 4.16 show the TMP flux curves for MBR 1 and MBR 2 respectively. The upward and downward arrows as seen on the graphs indicate the point of increase and decrease of fluxes. As observed from the curves, the critical flux for both MBR 1 and MBR 2 was found to be 10 LMH. The critical flux based on TMP increase is defined as the flux below which the fouling does not occur. Therefore, when the TMP flux curve deviates from a straight line, fouling was observed. Both stages were at a similar MLVSS concentration when the tests were carried out, around 4,700 – 5,440 mg/l. For MBR 2, the first test run was started at 10 LMH and increased to 16 LMH before the flux was stepped down steadily. The second test was started at a much lower flux of 4 LMH. By comparing the two curves, it could be seen that fouling exhibited the same behaviour up to 14 LMH when critical flux (10 LMH) was exceeded. However, there was a sharp TMP rise when flux was increased to 16 LMH. Figure 4.16 also shows that a significant hysteresis cycle was observed as compared to the test started at a lower flux of 4 LMH. This showed that a different fouling history affects the subsequent fouling behaviour.

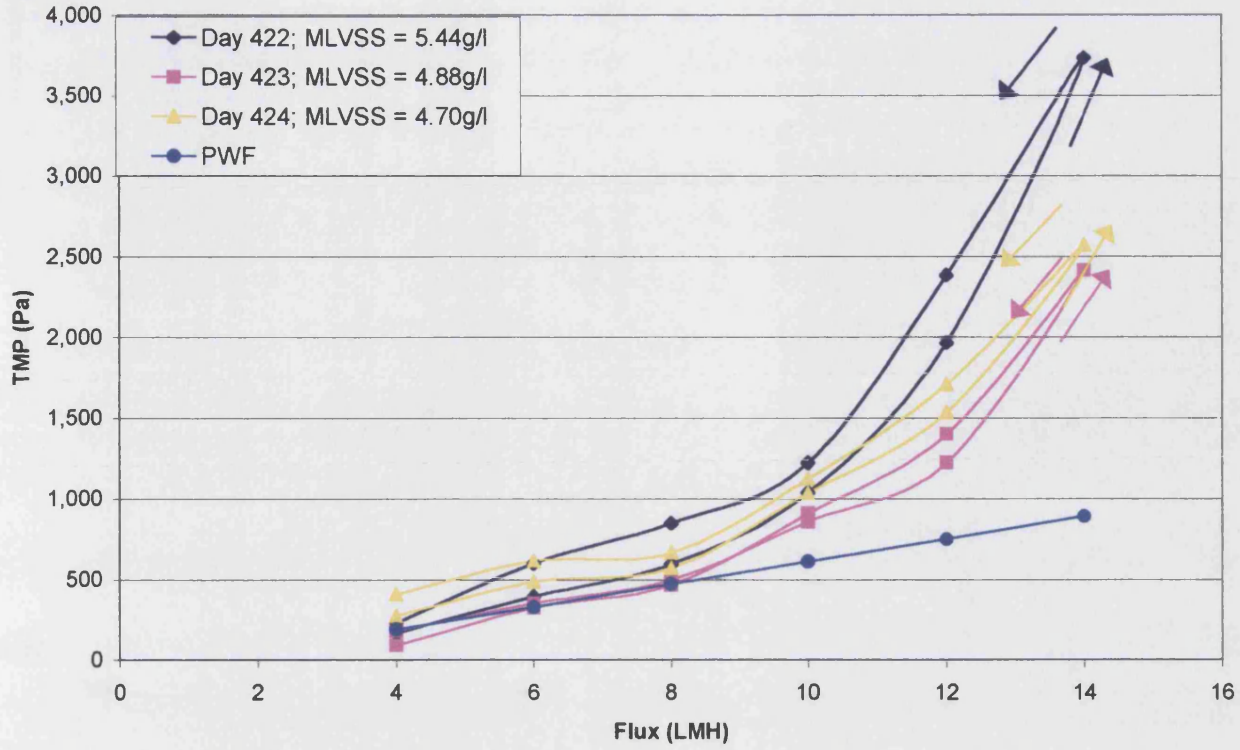


Figure 4.15: TMP against flux plot for MBR 1 to determine critical flux

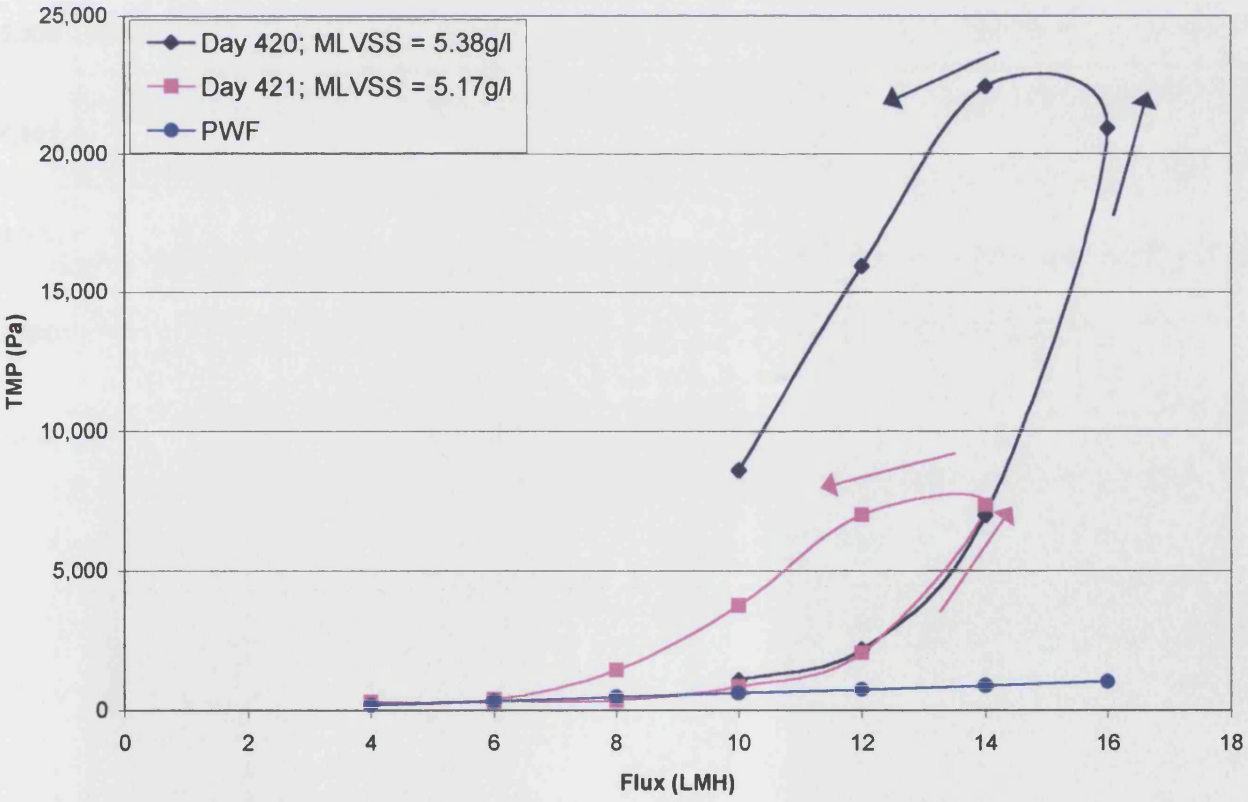


Figure 4.16: TMP against flux plot for MBR 2 to determine critical flux

Figure 4.17 shows a comparison of the TMP flux curves for MBR 1 and MBR 2. It can be observed that for MBR 1, fouling showed slight hysteresis. MBR 2 however showed a much more severe fouling when the critical flux had been exceeded, as seen from the bigger hysteresis cycle. Even though the two MBRs were at similar MLVSS concentrations when the tests were carried out, they did not exhibit the same fouling characteristics. This shows that the activated sludge composition, which will be different in each MBR, affects the fouling nature. Also, it may be concluded that the sludge in MBR 2 showed a tendency to a higher degree of fouling when the critical flux was exceeded. This could be due to the role of MBR 2 in cell degradation and lysis, which led to a sludge nature that is prone to a higher degree of fouling.

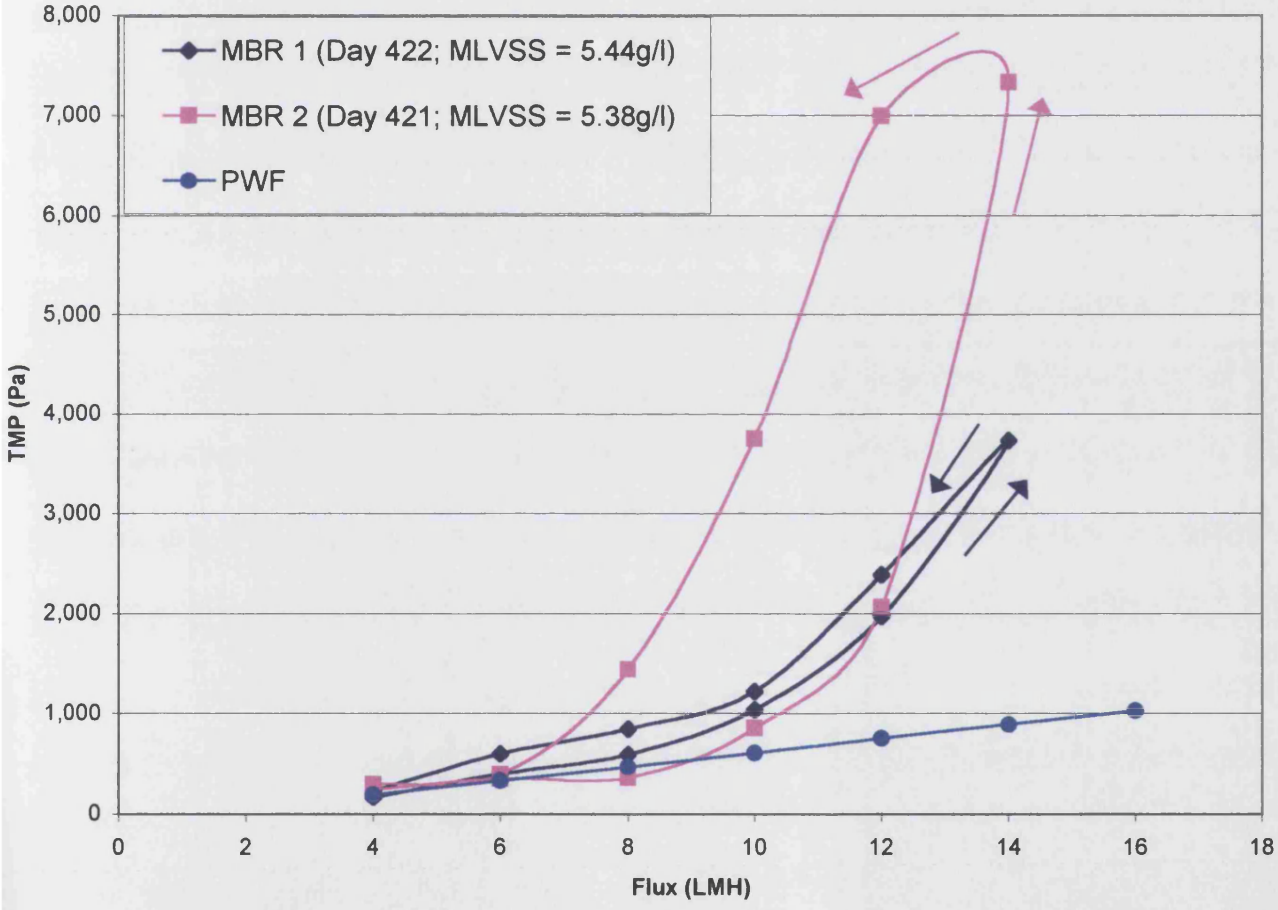


Figure 4.17: TMP against flux curves for MBR 1 and MBR 2 to compare the hysteresis effects

4.7 Conclusions

The experimental findings reported in this chapter are promising and shows the plausibility of the two-stage MBR concept, where MBR 1 is focused on maximal organic degradation and MBR 2 is focused on minimum sludge yield / production. In this case, MBR 1 operated at 6 h HRT and 16 d SRT, while MBR 2 operated at 19 d HRT and 100 d SRT throughout the analytical and observation period of 424 days. MBR 1 yielded satisfactory performance in terms of organic degradation, as indicated by the excellent permeate quality and organic removal rate. MBR 2 demonstrated an overall 60 % reduction in the sludge production in comparison to a single MBR system. The results are summarised as the following:

1. Permeate of MBR 1 meets the stringent discharge limits with approximate BOD_5 and COD values of 4 mg BOD_5/l and 27 mg COD/l.
2. The excellent BOD_5 and COD removal rates of MBR 1 (98% for BOD_5 reduction and 91 % for COD reduction) are unrivalled by the conventional activated sludge (AS) process.
3. Two-stage MBR gives a much lower sludge yield of 0.13 kg MLSS/kg BOD, in comparison to other wastewater treatment processes such as BAF, trickling filter, conventional AS.
4. The two-stage MBR offers an overall sludge reduction of 62.2% in comparison to the single MBR 1.
5. The two-stage MBR configuration has a lower volumetric sludge production rate compared to the single stage MBR 1, which leads to substantial economic savings on the overall sludge disposal and treatment cost.
6. The volumetric COD removal rate of MBR 1 was overall 70 % higher than MBR 2. These results are in accordance to the proposed concept for the two-stage MBR configuration, where the first stage is focused on high COD / BOD removal rate and the second stage is focused on reduced sludge production rate.
7. MBR 1 has a higher SOUR value than MBR 2. This indicates that MBR 1 has a higher biological activity than MBR 2, due to the biomass being more viable and actively metabolising the synthetic feed in MBR 1; while MBR 2 is more focused on cell lysis and endogenous respiration.

8. Permeate of MBR 2 also shows excellent BOD_5 and COD values of approximately 12 mg BOD_5/l and 59 mg COD/l, which are well below the discharge limits.
9. MBR 2 shows a gradual pH drop due to nitrification and experienced disturbance from alga bloom. This led to the start-up of a smaller scale new MBR 2. The new MBR 2 also shows a gradual pH drop since start-up.
10. The nitrogen tests show that there was nitrification in the MBRs. However, the nitrification from ammonia to nitrate was inhibited in MBR 1, as shown by the build up of intermediate nitrite concentration in permeate of MBR 1.
11. For both the original and the new MBR 2, there was a high concentration of ammonia present in the permeate due to cell lysis into organic nitrogen matter. The high nitrate and low nitrite concentration shows a high nitrification activity.
12. The nitrogen concentration in the permeate streams of MBRs did not meet the discharge limits. The nitrogen load in the synthetic feed recipe is higher in organic form (protein present in peptone and meat extract) which is unstable and subject to further decomposition, compared to real wastewater effluent that has higher inorganic nitrogen content in the form of urea. Therefore, the degradation of the organic nitrogen by bacteria releases more ammonia nitrogen, which is then converted into nitrite and nitrate, leading to higher nitrogen concentrations in the permeate streams. However, if real sewage is employed in the MBR, the performance of the MBR would be expected to be improved.
13. Nitrification could be inhibited due to limited oxygen concentrations and non-optimal pH conditions. Therefore, it is necessary to ensure that dissolved oxygen concentration is not limiting and alkalinity control is employed to enhance nitrification. Alternative process design (including a separate nitrification / denitrification stage) and a different operational parameter (longer SRT) would possibly improve the quality.
14. The fouling tests show that the critical flux determined for both MBR 1 and MBR 2 was 10 LPM. However, the TMP against flux graphs show that the MBRs do not exhibit the same fouling characteristics, as seen from the different hysterisis effects, even though the tests were carried out at similar MLVSS concentrations. The sludge in MBR 2 showed a tendency for a higher degree of fouling when the critical flux was exceeded. The critical flux information for the MBRs is crucial in

designing the operation parameters to allow the MBRs to operate for a long period of time with minimum fouling.

Chapter 5: Respirometry as a tool for determination of kinetic parameters for MBR 1 and MBR 2

Scope of Chapter 5

Respirometry has been a useful tool in the determination of the kinetic parameters for the mathematical modelling of activated sludge. This chapter reviews the batch respirometric studies and the resulting kinetic constants reported within the literature. OxiTop®, WTW was used as a tool for respirometric studies in an attempt to determine the kinetic parameters for the MBRs. Different problems encountered with the technique are outlined and conclusions are drawn about the use of the batch respirometric studies.

5.1 Introduction

The advances and progress in the mathematical modelling of activated sludge have been one of the major breakthroughs in assisting the mechanistic understanding of the system and the prediction of process performance. However, the merit of models mainly depends on the accuracy and reliability of the information they reflect on the wastewater to be treated and the biochemical mechanisms involved. The different aspects to be determined via experimental method include (i) wastewater fractions included as model components; (ii) biomass fractions in activated sludge and wastewater, and (iii) various kinetic and stoichiometric parameters defining biochemical processes in the models (Insel *et al.*, 2002).

The kinetic parameters for activated sludge were classically determined with traditional approaches, which have been replaced by batch rapid respirometric studies in recent years due to difficulties either in the interpretation of the results or the time-consuming procedures. For example, the maximum specific growth rate for heterotrophs (μ_H) was traditionally determined on the basis of the relationship between the specific growth rate or the specific substrate removal rate and the substrate concentration, usually reflected by a convenient linearized form of the Monod kinetic equation, using overall VSS and COD measurements to define biomass and substrate (Williamson and McCarty, 1975). The difficulties in the

interpretation of COD and VSS measurements encouraged the development of experimental procedures using respirometry for the assessment of kinetic constants.

5.2 Theoretical background of the kinetic equations

5.2.1 Determination of μ_m and K_s

Using respirometry, the data could be collected much more efficiently than any other alternative methods as it could be collected automatically and in greater quantities. The respirometric data generated is then translated into either biomass growth data or substrate utilisation data, which are then analysed for the determination of biokinetic growth constants, μ_m and K_s. According to the Monod growth equation,

$$\mu = \frac{\mu_m S}{K_S + S} \qquad (5.1)$$

where μ = specific growth rate of biomass, h^{-1}

μ_m = maximum specific growth rate of biomass, h^{-1}

S = substrate (waste) concentration, mg COD/l

K_s = half-saturation substrate concentration, mg COD/l

Therefore, a set of specific growth rate and substrate (waste) concentration data, i.e., μ and S, are required to fit the Monod equation to determine the biokinetic constants. Once μ and S are correlated, the model calibration is complete.

In this study, the respirometric data measured as cumulative oxygen uptake rate was converted into biomass growth data linked by the following mass balance equation for substrate removal in aerobic biological systems. Based on the assumption that the COD being removed from the solution during metabolism is channelled in varying proportion into the synthesis of new cells (anabolism) and to respiration (catabolism) measurable as oxygen uptake rate, this is quantified into Equation 5.2.

ΔCOD	=	Oxygen uptake	+	ΔCOD_{cells}	(5.2)
(Substrate utilisation)		(Respiration)		(cell synthesis)	

Equation 5.2 represents the complete mass balance for substrate removal in aerobic biological systems under the assumption that it is in the absence of any physical stripping and chemical oxidation of the substrate. The amount of substrate COD channelled to biomass (ΔCOD_{cells}) can be expressed as the product of the amount of cells produced (ΔX) and the unit COD of the cell mass (O_x), given by Equation 5.3.

$$\Delta COD_{cells} = \Delta X \, . O_x \qquad (5.3)$$

The expression of substrate utilisation, ΔCOD is given by the equation for cell yield

$$Y = \Delta X / \Delta COD \qquad (5.4)$$

and therefore

$$\Delta COD = \Delta X / Y \qquad (5.5)$$

Recognizing that the amount of cell produced, $\Delta X = X_t - X_0$ (where X_t and X_0 are X at some time t and at time 0, respectively) and substituting Equations 5.3 and 5.5 into Equation 5.2,

$$\frac{(X_t - X_0)}{Y} = O_2 \ \ uptake + (X_t - X_0) \ O_X \qquad (5.6)$$

Rearranging and simplifying Equation 5.7 yields

$$X_t = X_0 + \frac{O_2 \ \ uptake}{(1/Y - O_X)} \qquad (5.7)$$

Equation 5.7 is used to convert the generated oxygen uptake data into biomass growth curves (each growth curve is generated for each respirometric unit), which can be analyzed to give rise to specific growth rate (μ) at different initial substrate concentrations (S_0). Selecting a value for the cell yield, Y, and cell COD, O_X is a prerequisite to using the Equation 5.7. These parameters can be determined from actual test data or estimated using previous information on the particular waste treatment system from past kinetic studies. In this case, the cell yield for each of the

MBRs was calculated from the experimental data using Equation 5.4. Cell COD, O_X, can be calculated using Equation 5.8, where

$$O_X = \frac{(Total\ COD - Soluble\ COD)}{X\ or\ MLVSS} \qquad (5.8)$$

The average O_X value was found experimentally to be 1.45, which is very close to the suggested value of 1.42 mg COD/mg X according to Porge's stoichiometric formula for activated sludge cell ($C_5H_7NO_2$) (Rozich and Gaudy, 1992).

5.2.2 Determination of endogenous decay rate, k_d

The oxygen consumption rate can be corrected for activated sludge concentration as follows:

$$\frac{dO}{dt} = 1.42\ k_d X \qquad (5.9)$$

The endogenous decay rate, k_d, is defined as the rate of cell mass decrease per unit of mass:

$$k_d = -\frac{dX}{Xdt} \qquad (5.10)$$

which can be integrated into

$$X_t = X_0 e^{-k_d t} \qquad (5.11)$$

where X_t = cell mass at time t, mg VSS/l

X_0 = initial cell mass, mg VSS/l

Substituting Equation 5.11 into Equation 5.9 yields

$$\frac{dO}{dt} = 1.42\ k_d X_0 e^{-k_d t} \qquad (5.12)$$

Taking the natural logarithm, Equation 5.12 becomes

$$\ln\left(\frac{dO}{dt}\right) = \ln(1.42\ k_d X_0)\ k_d t \qquad (5.13)$$

In Equation 5.13, k_d is the slope of the ln(dO/dt) vs. time plot. The dO/dt is the rate of oxygen consumption and the data can be generated by an electrolytic respirometer.

5.3 Respirometry

Respirometry is the measurement and interpretation of the biological oxygen uptake rate of activated sludge, based on the knowledge of the electron acceptor, under well defined experimental conditions (Spanjers *et al.*, 1999; Gernaey *et al.*, 2001). It has long been a useful tool in the field of water pollution research due to its rapid and simple operation. It provides a mean for researchers to gain knowledge about activated sludge biodegradation (stoichiometric and kinetic coefficients) and wastewater characteristics. In recent years, respirometry of activated sludge has proved a popular tool in model calibration such as the well-established IAWQ Activated Sludge Model No. 1, 2 and 3 (Spanjers and Vanrolleghem, 1995; Spanjers *et al.*, 1999; Vanrolleghem *et al.*, 1999). The respiration rate is often expressed as the oxygen uptake rate per unit of volume and time (mg O_2/l.min).

The respiration rate is measured with respirometers using different measuring principles. Generally, respirometers can be divided into two groups: closed respirometers and open respirometers. Here, the author will look into the static gas-static liquid method in more detail. This method was employed due to the equipment that was available for the respirometric tests (OxiTop®, WTW). Furthermore, it offers the advantage of easy operation and its usage was reported by different researchers in the literature (Cech *et al.*, 1984; Kappeler and Gujer, 1992; Kristensen *et al.*, 1992; Ubay Cokgör *et al.*, 1998). The readers are recommended to look into the literature (Milenko, 1993; Gernaey *et al.*, 2001) for other types of measuring principles and respirometers.

A static gas-static liquid respirometer is typically operated by monitoring the decline in dissolved oxygen concentration S_{O2} with time in a closed vessel after a short

aerated phase (Gernaey *et al.*, 2001). The danger with closed system studies is that due to absence of aeration, oxygen limitation might occur. A way to solve this is to oversaturate the activated sludge and test samples with pure oxygen to achieve a higher initial dissolved oxygen concentration (Ellis *et al.*, 1996). In this study, the OxiTop®, WTW was employed to measure the oxygen uptake rate. The measuring principle will be discussed further in the methodolody section later.

5.3.1 Batch respirometric studies

Batch respirometric studies are generally used for determination of wastewater and sludge charateristics (Henze, 1992; Kappeler and Gujer, 1992; Chudoba *et al.*, 1992b; Spanjers and Keesman, 1994; Vanrolleghem *et al.*, 1994) because they are simpler to operate and problems and interferences inherently associated with reactor hydraulics are avoided. Continuous experiments are useful for determination of stoichiometric coefficients, however they require a long measuring period since steady state must be attained.

The most important parameter in the batch experiments, as demonstrated by the work of Chudoba *et al.* (1992b), is the ratio of the initial substrate concentration (S_0) to the initial biomass concentration (X_0), known as S_0/X_0. A high S_0/X_0 ratio results in substantial cell multiplication and growth. This causes a shift in biomass population and changes the proportion among slow-growers and fast-growers so that the obtained kinetic parameters are no longer representative of the original system (Novák *et al.*, 1994a). Hence, for biodegradation studies aiming to obtain kinetic parameters, it is necessary to work at low S_0/X_0 ratios to prevent mixed culture microorganisms from substantial multiplication. However, the threshold between low and high values of S_0/X_0 ratio is not defined strictly. It was suggested that interval between 2 and 4 would be a correct estimation according to Speece *et al.* (1973), Pitter and Chudoba (1990) and Chudoba *et al.* (1991b). Nevertheless, there are batch kinetic studies that were carried out with a much lower S_0/X_0 ratio by Spanjers and Vanrolleghem (1995), at 1/200 and 1/20 on a COD basis.

One of the pioneering studies in the field was the method proposed by Ekama *et al.* (1986), where aerobic or anoxic reactors were run under suitably low initial S_0/X_0

ratios (0.2 – 0.8 g COD/g VSS), based on the assessment of readily biodegradable substrate. Later on, Kappeler and Gujer (1992) suggested a batch respirometric study based on high initial S_0/X_0 ratios (4 – 5 g COD/g VSS), for the assessment of the growth constants. Their method was criticised by Novák *et al.* (1994a), on the basis that it is likely to create a microbial growth medium totally different from conditions associated with activated sludge growing in continuous systems; as batch reactors would favour the fast growing organisms and eventually lead to a unrealistically high μ_H value. This will be discussed further in the results and discussion section for MBR 1 (see Section 5.5.1).

5.3.2 Reported literature values for maximum specific growth rate, μ_m and saturation constant, K_s

Orhon and Artan (1994) summarized significant experimental work previously carried out with conventional approach using only two overall parameters of substrate (BOD or COD) and biomass (VSS) and this is shown in Table 5.1. In addition to that, Table 5.2 lists the growth constants reported in the literature, either measured by similar batch respirometric techniques or adopted for model simulation.

Table 5.1: Reported values of μ_H for domestic sewage determined with conventional approach.

μ_H (d^{-1})	Basis for constant	Reference
0.60	BOD_5	Pearson (1968)
1.70	COD	Pearson (1968)
6	BOD_5	Lawrence and McCarty (1970)
3.75	COD	Lawrence and McCarty (1970)
1.43 – 13.2	BOD_5	San (1992)
3.2 – 3.75	COD	San (1992)

Table 5.2: Heterotrophic growth constants reported in the literature.

Wastewater	T (°C)	μ_H (d^{-1})	K_S (mg. l^{-1})	Reference
Domestic sewage	20	6	20	Henze *et al.* (1987)
	10	3	20	Henze *et al.* (1987)
Domestic sewage	-	4	5	Billing and Dold (1988)
Domestic sewage	20	1.50	5	Sollfrank and Gujer (1991)
	10	0.75	5	Sollfrank and Gujer (1991)
Synthetic	-	4	5	Gujer and Henze (1991)
Synthetic	20	6	5	Larrea *et al.* (1992)
Domestic sewage	20	5	10	Kappeler and Gujer (1992)
Domestic sewage (θ_C = 2 day)	22	2	3.5	Kappeler and Gujer (1992)
Domestic sewage (θ_C = 10 day)	22	7	3.5	Kappeler and Gujer (1992)
Domestic sewage (θ_C = 2 day)	13	1	4	Kappeler and Gujer (1992)
Domestic sewage (θ_C = 11 day)	13	2	4	Kappeler and Gujer (1992)
Domestic sewage (θ_C = 4 day)	15	1.5	2.5	Kappeler and Gujer (1992)
Domestic sewage	20	3	20	Sorour *et al.* (1993)
Domestic sewage	20	2.5	11	Stokes *et al.* (1993)
Domestic sewage	20	2.5	5	Siegrist *et al.* (1995)
Domestic sewage	20	6	5	De la Sota *et al.* (1994)
Synthetic	-	2 – 2.5	2	Novák *et al.* (1994b)
Domestic sewage	20	6	4	Henze *et al.* (1995)
	10	3	4	Henze *et al.* (1995)
Domestic sewage	-	3.4 – 6.5	-	Sözen *et al.* (1998)

Note: θ_C is the sludge age, also known as SRT.

5.3.3 Endogenous respiration / decay rate, k_d

The conventional way to determine biomass yield and k_d was by operating a few sludge ages and using the linearised form of Monod kinetics. However, this method is

time-consuming and therefore not rapid. Also the traditional approaches for the assessment of endogenous decay rate, k_d, based upon the measurement of volatile suspended solids (VSS), have caused much confusion and inconvenience to the basic understanding of activated sludge behaviour. This is mainly due to the inability of the VSS parameter to differentiate between active biomass, inert particulate matter and inert residual products presumably generated from endogenous respiration. With the development of the multi-component models, a new concept of aerobic endogenous decay rate, b_H, has been introduced, solely associated with active heterotrophic biomass established in the IAWQ Task Group Model (Henze *et al.*, 1987). Marais and Ekama (1976) also proposed a new respirometric method for the assessment of b_H under aerobic conditions. Despite its significance in the modelling of activated sludge, the endogenous decay process remains the area that receives the least experimental support, both in terms of method of assessment and available data readily and reliably usable in modelling.

Table 5.3: b_H values as reported in the literature for domestic wastewaters.

Sludge age (d)	Temperature (°C)	b_H (d^{-1})	Reference
	20	0.24	Sollfrank and Gujer (1991)
	10	0.12	Sollfrank and Gujer (1991)
2	20	0.25	Kappeler and Gujer (1992)
10	22	0.1	Kappeler and Gujer (1992)
2	22	0.4	Kappeler and Gujer (1992)
11	13	0.05	Kappeler and Gujer (1992)
4	13	0.1	Kappeler and Gujer (1992)
	15	0.08	Kappeler and Gujer (1992)
	15	0.11	Sarikaya *et al.* (1997)
	20	0.24	Marais and Ekama (1976)
	14	0.20	Warner *et al.* (1983)
	20	0.24	Henze *et al.* (1987)
	10	0.077	Henze *et al.* (1987)
	20	0.153	Henze *et al.* (1995)
	10	0.077	Henze *et al.* (1995)
	20	0.09	Avcioglu *et al.* (1998)

5.4 Methodology and procedures

5.4.1 Equipment

The OxiTop® Control System (WTW, Germany) was used for the batch respirometric kinetics studies of the MBRs. The data recorded by the piezoelectric pressure head was converted into the cumulative oxygen uptake rate and used to calculate the growth kinetics of the microbial system.

5.4.2 Procedures

The sample wastewater solution is seeded by washed activated sludge taken from the MBRs and added to each OxiTop® BOD bottle. Each bottle is prepared at a different food to microorganism (F/M or S_0/X_0) ratio, and contains a different initial wastewater concentration (S_0). The activated sludge needed to be washed in order to remove any soluble and adsorbed substrate. No nitrification inhibitor was used in these tests and therefore the total OUR measurement is the sum of the heterotrophic and autotrophic OUR. This was in reference to the work of Novák *et al.* (1994a) as they also carried out their batch respirometric tests without a nitrification inhibitor. This is in accordance with the conclusions of Dold *et al.* (1991) that thiourea could inhibit not only nitrification and hydrolysis but also the activity of microorganism respiration on readily biodegradable substrate. Also, Novák *et al.* (1994a) demonstrated that in the calculation of the maximum specific growth rate, the influence of autrotrophic OUR is negligible.

5.4.2.1 Kinetic studies for MBR 1

To determine the kinetics for MBR 1, the synthetic feed was used (refer to Chapter 3 for the recipe). 70 ml of activated sludge from MBR 1 was withdrawn and aerated overnight in a bottle. The sludge was washed by adding 50 ml of dilution water containing all the essential salts present in the OECD synthetic feed recipe except of the carbon source such as meat extract, peptone and urea (refer to Chapter 3). The dilution water had also been aerated overnight. The solution was mixed gently in the centrifuge bottle and centrifuged at 3,000 rpm for 2 minutes to separate the supernatant from the cells. The supernatant was poured away and 50 ml of dilution water was added. The washing procedure was repeated for 3 times and the final 60 ml of activated sludge was now washed and ready to be used for the respirometry tests.

A total amount of 240 ml of solution made out of sludge, dilution water and substrate was prepared for each respirometric test.

Table 5.4: Different proportion of substrate, dilution water and sludge for the kinetic studies test of MBR 1.

Bottle	A	B	C	D	E	F
Substrate (ml)	30	60	110	170	200	-
Dilution water (ml)	200	170	120	60	30	230
Sludge (ml)	10	10	10	10	10	10

240 ml solution mixture of sludge, dilution water and substrate was prepared according to Table 5.4. There are 6 bottles, each with a different F/M ratio, corresponding to a range of 0.014 and 1.775. Once the solution was prepared, it was poured into a 167 ml overflow flask designated for the BOD range of 0 – 400 mg/l. The content was then poured into the BOD bottle and the same procedure as that for BOD_5 determination was followed, whereby oxygen uptake was monitored (see Chapter 3). The results were analysed with an EXCEL spreadsheet and the calculated specific growth rate (μ) and initial substrate concentration (S_0) results were fitted to the Monod model by non-linear least squares method with CurveExpert 1.3 (a statistical computer software), for the parameter estimation of the biokinetic constants μ_m and K_s (see Figure 5.2 in Section 5.5).

Cell F with no substrate and only dilution water was to be used to determine the endogenous decay rate, k_d.

To determine the mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentration used for the test, Whatman glass fibre GF/C filter papers were dried in a furnace at 550 ± 50 °C for 1 hour to get rid of any volatile compounds. The papers were placed into a desiccator and the weight of the empty papers was noted. A Whatman filter unit was assembled and used for the filtration of mixed solution prepared for each cell. 30 ml of sample was pipetted and filtered through the filter papers. A duplicate was used for every cell. The filter papers were

then evaporated and dried to constant weight in an oven at 103 – 105 °C to determine MLSS. After noting the weight of the filter papers, the papers were ignited in the furnace at 550 ± 50 °C for 1 hour for MLVSS determination. The filtrate was used for COD and TOC analysis.

5.4.2.2 Kinetic studies for MBR 2

The attempt to determine kinetic parameters for MBR 2 was made complicated by the fact that it was difficult to find a suitable substrate to be employed in the tests. Various substrates were tested. As MBR 2 did not receive a constant supply of synthetic feed as a food source, it was proposed that different metabolism than that of MBR 1 prevailed. Hence, both activated sludge from MBR 1 and 2 as well as permeate from MBR 1 was used as a substrate in an effort to find the kinetics of MBR 2. It was proposed that cryptic growth is one major phenomenon occurring in MBR 2 and hence the theory of using lysis products from sonicated sludge as possible substrates for the microbial populations in MBR 2.

35 ml of sludge from MBR 2 was diluted by half with dilution water and aerated overnight. 2 litres of dilution water was prepared and aerated overnight. 60 ml of dilution water was added to each of the two centrifuge tubes containing 15 ml of sludge each. The tubes were centrifuged at 4,000 rpm for 4 minutes and the supernatant was poured away. The washing procedure was repeated three times and the remaining 30 ml sludge was used for the test. 240 ml mixed solution of substrate, sludge and dilution water was prepared according to the following composition for each cell in Table 5.5.

Table 5.5: Different proportion of substrate, dilution water and sludge for the kinetic studies of MBR 2.

Cell	A	B	C	D	E
Substrate (ml)	-	20	40	55	75
Dilution water (ml)	235	215	195	180	160
Sludge (ml)	5	5	5	5	5

The substrate or lysate was prepared as follows. 210 ml of sludge from either MBR 1 or MBR 2 was sonicated in a beaker with Grant XB6 Ultrasonic Bath for 30 minutes. The solution was then centrifuged at 7,000 rpm for 20 minutes. The supernatant was withdrawn with a disposable syringe and filtered through Whatman GF/C filter paper. The lysis product / solution obtained after filtration was used as the substrate to find the kinetics of MBR 2. The MLSS and MLVSS concentrations for each cell were found by following the same procedures as for MBR 1 kinetic determination. In a similar way, the results were analysed and fitted to the Monod model to determine μ_m and K_S. Cell A with no substrate and only dilution water was to be used to determine the endogenous respiration rate, k_d.

5.5 Results and Discussions

Figure 5.1 shows an example of the logarithmic biomass curve against time based on results obtained for MBR 1. For consistency and comparability of the results, the calculations of the specific growth rate were taken from t = 1 to 12 h, where the exponential growth of the biomass could be observed.

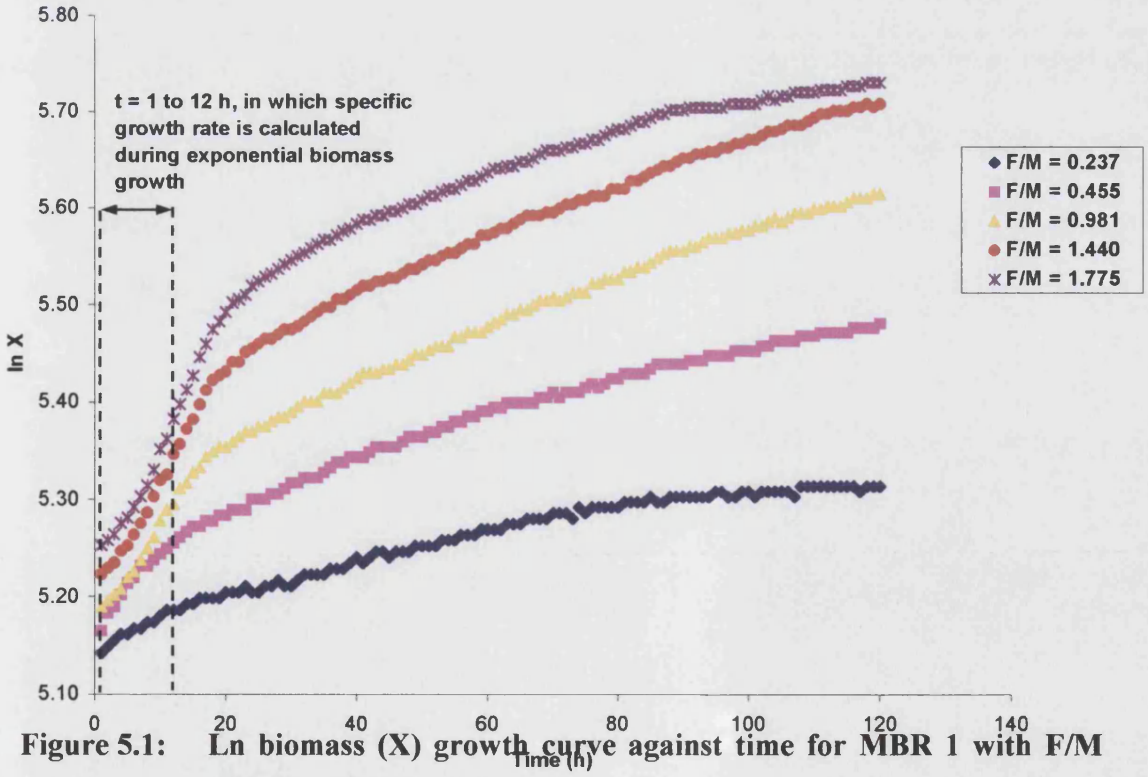
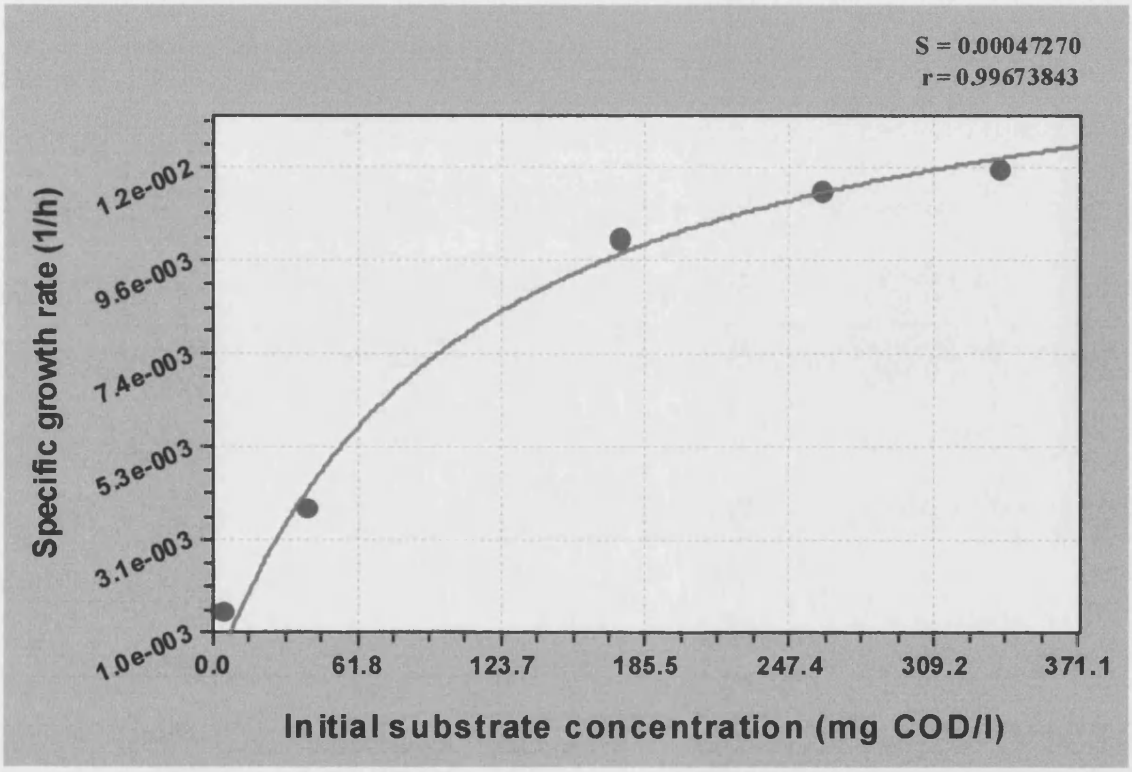


Figure 5.1: Ln biomass (X) growth curve against time for MBR 1 with F/M ratio between 0.237 and 1.775

The specific growth rate data, μ obtained for each biomass curve are then plotted against substrate concentration, S (μ vs. S) with a computer curve fit software. The author used the CurveExpert version 1.37 for fitting the experimental data to the Monod function according to Equation 5.1 using a nonlinear least squares method to determine the biokinetic constants. Figure 5.2 shows an example of the plot based on results obtained for MBR 1. The curve fit analysis yielded values of 0.016 h^{-1} and 108.06 mg COD/l for μ_m and K_s, respectively. The plot showed that the Monod equation provided a reasonable description of the experimental data as evidenced by the low standard error (S) value of 0.00047 and high correlation coefficient (R^2) of 0.997.



Note: S is the standard error value and r is the correlation coefficient.

Figure 5.2: Plot of specific growth rates, μ (h^{-1}) against substrate concentration, S (mg COD/l) from a computer curve fit analysis of the Monod function according to Equation 5.1. (● Experimental data, —— Curve fit)

5.5.1 Estimation of μ_m and K_s for MBR 1

The biokinetic growth constants, μ_m and K_s, are the biological parameters having the most influence on predicting treated effluent quality. Therefore, an accurate determination of these parameters is important for model calibration. However, they are also the ones more likely to exhibit the greatest level of variation with changing environmental conditions (Rozich and Gaudy, 1992). Hence, μ_m and K_s values are likely to differ from one system to another.

The batch respirometric kinetic studies for MBR 1 were carried out at an F/M or S_0/X_0 ratio between 0.014 and 1.775. Preliminary tests at different F/M ratios had also been carried out. However, Chudoba *et al.* (1992b) showed that the results for the kinetic studies were only comparable on the basis of a similar F/M ratio. Therefore, the results of the following tests that gave more comparable results are reported here.

The kinetic parameters, μ_m and K_s were obtained for MBR 1 based on two different yield values. One of them is the experimental yield from the continuous operation of MBR 1, which is 0.25 g SS/ g COD while the other is a reported yield of 0.65 g SS/ g COD (Metcalf and Eddy, 1991). Both sets of results are tabulated and plotted as shown by Tables 5.6 – 5.9 and Figure 5.3 and 5.4. Also the results based on calculation of the substrate expressed in mg COD/l or mg BOD/l are shown.

From Table 5.6, the average values of μ_m and K_s were $0.018 \pm 0.004\ h^{-1}$ and 145.31 ± 61.64 mg COD/l. The curve fit analysis yielded reasonable values for μ_m and K_s as evidenced by the low standard error (S) and high correlation coefficient (R^2). However, when a higher sludge yield (Y = 0.65) was used, μ_m found was an order of magnitude higher while K_s was much smaller. The results are tabulated in Table 5.7. The average values for μ_m and K_s were $0.150 \pm 0.011 h^{-1}$ and 38.82 ± 6.33 mg COD/l. In addition, the curve fit analysis showed higher standard errors (S) and lower correlation coefficients when a higher yield is used. The reported values for μ_m and K_s in the literature are tabulated in Tables 5.1 and 5.2 in Section 5.3.2. A comparison showed that the values calculated with a higher yield are closer to the reported literature values. However, the readers have to bear in mind that μ_m and K_s are very likely to exhibit the greatest level of variation with changing environmental

conditions (Rozich and Gaudy, 1992). Hence, μ_m and K_s values are likely to be case sensitive and differ from one system to another. This is also supported by Sözen *et al.* (1998), in which they concluded from their findings and a comparison of literature reported values that μ_H is quite site-specific and model-specific and may be affected by compounds in sewage with an inhibitory effect. Also, the results for μ_m were comparable for both cases of yield even when the substrate is expressed in mg BOD/l. But the results for K_s were smaller when substrate is expressed in mg BOD/l. It could be observed that K_s calculated with a smaller yield showed more scattering than K_s calculated with a larger yield for both cases of substrate expressed in COD or BOD.

Table 5.6: μ_m and K_s (mg COD/l) of MBR 1 determined from batch respirometric studies (calculated with Y = 0.25). *

Days of Operation	μ_m (h^{-1})	K_s (mg COD/l)	Standard error, S	Correlation coefficient, R^2
Day 304	0.023	229.02	0.00084	0.9922
Day 318	0.016	108.06	0.00047	0.9967
Day 324	0.018	153.10	0.00053	0.9956
Day 394	0.013	91.07	0.00053	0.9956
Average ± std. dev.	0.018 ± 0.004	145.31 ± 61.64	-	-

Table 5.7: μ_m and K_s (mg COD/l) of MBR 1 determined from batch respirometric studies (calculated with Y = 0.65). *

Days of Operation	μ_m (h^{-1})	K_s (mg COD/l)	Standard error, S	Correlation coefficient, R^2
Day 304	0.147	33.39	0.02067	0.9479
Day 318	0.153	38.09	0.01435	0.9749
Day 324	0.164	47.87	0.01334	0.9779
Day 394	0.137	35.93	0.00957	0.9512
Average ± std. dev.	0.150 ± 0.011	38.82 ± 6.33	-	-

** Note: MBR 1 at steady state, with operating conditions of HRT = 6 h and SRT = 16 d).*

Table 5.8: **μ_m and K_s (mg BOD/l) of MBR 1 determined from batch respirometric studies (calculated with Y = 0.25). ***

Days of Operation	μ_m (h^{-1})	K_s (mg BOD/l)	Standard error, S	Correlation coefficient, R^2
Day 304	0.024	148.57	0.00032	0.9989
Day 318	0.016	69.46	0.00034	0.9986
Day 324	0.019	97.53	0.00066	0.9956
Day 394	0.013	54.31	0.00047	0.9950
Average ± std. dev.	0.018 ± 0.005	92.47 ± 41.46	-	-

Table 5.9: **μ_m and K_s (mg BOD/l) of MBR 1 determined from batch respirometric studies (calculated with Y = 0.65). ***

Days of Operation	μ_m (h^{-1})	K_s (mg BOD/l)	Standard error, S	Correlation coefficient, R^2
Day 304	0.164	36.30	0.00500	0.9973
Day 318	0.163	32.93	0.00536	0.9970
Day 324	0.180	47.79	0.01076	0.9898
Day 394	0.137	21.44	0.00829	0.9882
Average ± std. dev.	0.161 ± 0.018	34.61 ± 10.84	-	-

**Note: MBR 1 at steady state, with operating conditions of HRT = 6 h and SRT = 16 d).*

The μ_m and K_s results are calculated based on the substrate expressed in both COD and BOD terms to find out the differences between the two expressions. The BOD results are calculated using the synthetic feed COD/BOD ratio, as reported in Chapter 4. In the literature, the results for K_s are reported either in the substrate expression of mg COD/l or mg BOD/l.

Due to limitations of equipment, replicates were not allowed as kinetics parameters such as k_d was also being determined in the same batch of experiments.

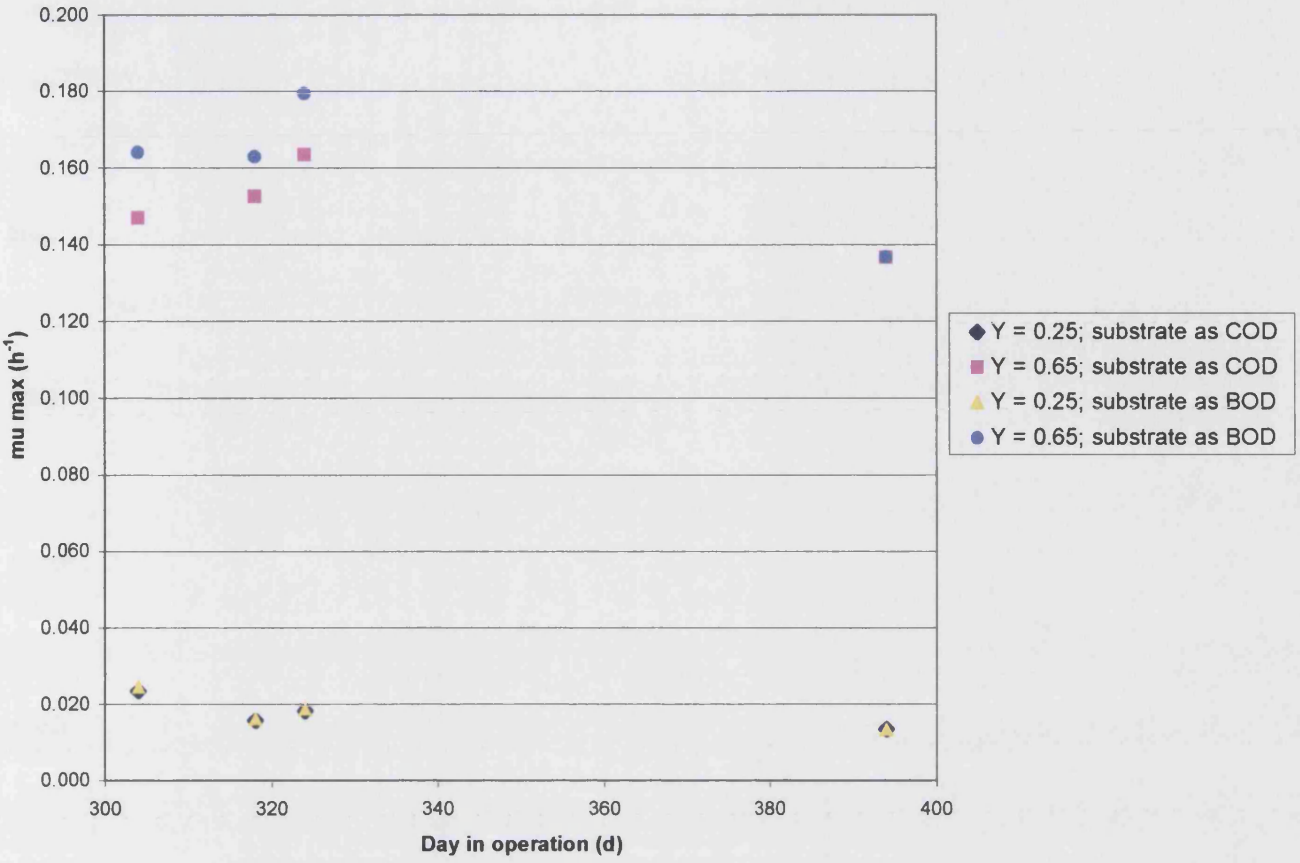


Figure 5.3: Comparison of μ_m of MBR 1 obtained from batch respirometric studies with different yield, Y and substrate expressed as BOD or COD. *

**Note: MBR 1 at steady state, with operating conditions of HRT = 6 h and SRT = 16 d).*

It can be seen that the kinetic parameters μ_m and K_s are very dependent on the value of cell yield. There arises a question as to which yield value should be used in the batch respirometric calculations in the determination of μ_m and K_s. In a batch respirometer, the biomass is subjected to an environment rich in substrate in order to promote exponential growth to determine the growth constants, μ_m and K_s. Hence, it can be argued that the biomass would have shifted towards a different physiological state compared to when it was still in the MBR, where there is limited food supply or a low F/M ratio was in operation. In the case of MBR 1, the F/M ratio was 0.35 g COD. g $MLVSS^{-1}$. d^{-1} and typical values for F/M ratios vary between 0.05 and 1.0 g COD. g $MLVSS^{-1}$. d^{-1} (Metcalf and Eddy, 1991). Pitter and Chudoba (1990) and Chudoba *et al.* (1992b) reported that the wastewater treatment plants generally work at very low

actual or available S_0/X_0 ratios; one or two orders of magnitude below the threshold between the low and high values of S_0/X_0 ratio used for respirometry given in the literature.

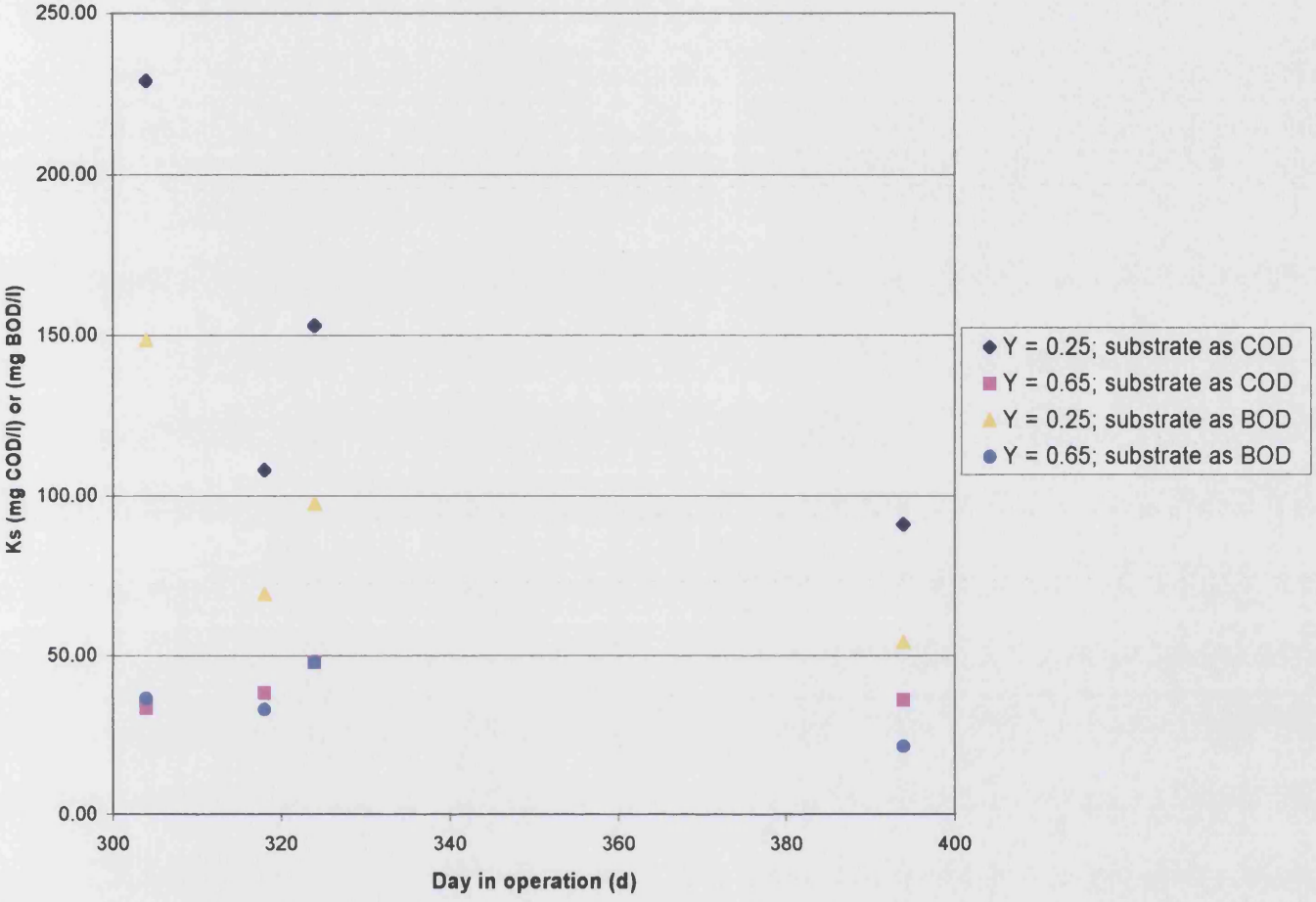


Figure 5.4: Comparison of K_s of MBR 1 obtained from batch respirometric studies with different yield, Y and substrate expressed as BOD or COD. *

**Note: MBR 1 at steady state, with operating conditions of HRT = 6 h and SRT = 16 d).*

Therefore, a higher value of yield would probably be found in a batch respirometer compared to the MBR due to a much higher S_0/X_0 ratio used in the tests. In the case of MBR 1, the batch respirometric kinetic studies were carried out at a F/M or S_0/X_0 ratio between 0.014 and 1.775 (compared to 0.35 in the MBR itself). But the question is which yield is the accurate one to be used for the calculation of the kinetic parameter? As can be seen that when Y = 0.65 was used, the standard error (S) were

higher and the correlation coefficient was lower from the curve fit analysis. Also is the batch respirometer the best method to study the kinetics of the system? Would the growth constants found from the batch respirometer with a high yield be representative of that of the biomass kinetics in the MBR itself?

Activated sludge is a consortium of many different microorganisms. Their growth rates differ from each other. Cultivation conditions determine which group prevails in the culture. The Monod equation is generally used for describing bacterial growth kinetics in terms of heterotrophic biomass under limiting substrate concentration. The application of the Monod kinetics on activated sludge biomass can strictly only be used under conditions where the biomass is considered to consist of only one species. For a continuously operated system at steady state, the assumption of equilibrium among different microorganism groups in the sludge can be assumed. Under these conditions, the Monod equation is supposed to be valid.

In a batch respitrometric study with the objective to obtain kinetic constants, Chudoba *et al.* (1992b) pointed out the importance of S_0/X_0. The S_0/X_0 ratio determines whether catabolism or anabolism prevails. At low S_0/X_0 ratios storage and accumulation phenomena without cell multiplication prevail. At higher S_0/X_0 ratios the microorganisms have enough substrate (energy) for their growth, and cell multiplication dominates and storage and accumulation phenomena are secondary. In other words, at low S_0/X_0 ratios the external substrate is exhausted before the cell multiplication can be detected. There are differing viewpoints in the literature and different researchers have carried out the respirometric studies under different S_0/X_0 ratios. Ekama *et al.* (1986) used moderate S_0/X_0 ratios (0.2 – 0.8 g COD/g VSS), whereas Kappeler and Gujer (1992) based their studies on high initial S_0/X_0 ratios (4 – 5 g COD/g VSS). As mentioned earlier on, Speece et al. (1973), Pitter and Chudoba (1990) and Chudoba *et al.* (1991b) suggested an interval between 2 and 4 to be a correct estimation. Nevertheless, there were batch kinetic studies that were carried out with a much lower S_0/X_0 ratio by Spanjers and Vanrolleghem (1995), at 1/200 and 1/20 on COD basis. This could explain the different μ_H and K_s results reported in the literature.

Another concern with batch respirometric studies for determination of kinetic constants was that sludge was taken from the MBR or its original cultivation conditions and being transferred into completely different growth conditions. As the cultivation conditions determine the proportion between each group of microorganisms present in the activated sludge biocenosis, this change can affect the microgranisms population quality and quantity, and thus preferences for certain microbial groups (Pitter and Chudoba, 1990; Chudoba *et al.*, 1992b).

Novák *et al.* (1994a) demonstrated that the maximum specific growth rates measured with simple batch techniques yielded a higher value compared to their combined technique of batch tests and computer simulation. Their new method estimates the proportions of autrotrophic and heterotrophic microorganisms in biomass by mathematical simulations. The maximum growth rates are then calculated from maximum oxygen uptake rates. They found more realistic values of the maximum specific growth rate of heterotrophic microorganisms (4 d^{-1}) compared to batch test cultivation (10 d^{-1}). They proposed a hypothesis that if two different microorganisms which significantly differ in their maximum specific growth rates were put into a batch test, this will affect which group will prevail. Under a continuously operated low loaded system, the group with lower growth rates would be favoured. However, under batch cultivation conditions with very high initial S_0/X_0 ratio, the group with a higher maximum growth rate will prevail. Therefore the major contribution to OUR will be from the faster growing group of microorganisms. They clearly demonstrated the changes in the activated sludge biocenosis during the batch test cultivation with high S_0/X_0 ratio and confirmed the hypothesis that during batch cultivation the microorganisms are exposed to different living conditions than in continuously operated systems. Batch cultivation conditions can change the proportion between groups of microorganisms from the activated sludge biocenosis by favouring fast growers under higher S_0/X_0 ratios. Consequently, the sludge parameters obtained in such a batch test can significantly differ from the parameters in a continuous flow system. This led to the conclusion the batch cultivation methods for the determination of kinetic and stoichiometric parameters for the purposes of mathematical modelling of biological wastewater treatment processes should be applied with caution.

Sözen *et al.* (1998) commented that the argument deserves consideration from a conceptual standpoint, but argued that this may be avoided by appropriate adjustment of the acclimatisation phase prior to the respirometric test. Their work, based on the adopted batch system, yielded reasonable μ_H values in the range of 3.4 – 6.5 d^{-1}, which was well in agreement of the reported literature data for domestic sewage (as tabulated in Table 5.2). Furthermore, Grady (1996) related this issue to culture history and suggested that conditions imposed before the kinetic test may have an impact on the measured parameters, therefore recommended that such impacts should be prevented by reducing the disturbances during the test and keeping the duration of the test relatively short.

In this work, μ_m estimated for MBR 1 was carried out at an F/M or S_0/X_0 ratio between 0.014 and 1.775, and the mean value calculated was 3.6 d^{-1} (assuming a yield of 0.65 and on the basis of COD for the calculation in the batch respirometric studies). The range of F/M or S_0/X_0 ratios chosen here is considered moderate. More importantly, the result of μ_m is reasonable when compared to the work of Sözen *et al.* (1998) on the basis of initial S_0/X_0 ratio. Sözen *et al.* (1998) reported μ_H values in the range of 3.4 – 6.5 d^{-1} for domestic sewage, carried out with S_0/X_0 ratios between 0.43 and 0.86. This result also compares well with the reported values of μ_H for domestic sewage using conventional approach (refer to Table 5.1), where Lawrence and McCarty (1970) found μ_H to be 3.75 d^{-1} and San (1992) found μ_H values of 3.2 – 3.75 d^{-1} based on the basis of COD. However, a much wider μ_H range of 1.5 – 7.0 d^{-1} is depicted as seen in Table 5.2, either measured by similar respirometric techniques or adopted for model simulation, which indicates clearly that μ_H is quite site-specific and model-specific and may be affected by compounds in sewage with an inhibitory effect (Sözen *et al.*, 1998). Metcalf and Eddy (1991) reported the range of K_s for the activated sludge process for domestic wastewater to be between 15 – 70 mg COD/l, with a typical value of 40 mg COD/l. The mean K_s determined for MBR 1 in this work (38.8 mg COD/l) is well in agreement with the reported value.

The values of μ_m and K_s determined for MBR 1 compare well with the reported values in the literature. This supported the validity of the results from this work. However, as mentioned earlier on, the batch respirometric studies were carried under

a higher F/M or S_0/X_0 ratio than that in MBR 1 (thus assuming a higher cell yield of 0.65 for calculation instead of 0.25 as experimentally determined for MBR 1). Thus, their application for mathematical modelling still needs to be verified (see Chapter 7).

5.5.2 Estimation of μ_m and K_s for MBR 2

Batch respirometric studies were also conducted for the determination of the kinetic parameters of MBR 2. Many attempts were made to find a suitable medium as a substrate for the biomass / inoculum from MBR 2 for the tests. As mentioned previously, MBR 2 was operated under no feed supply, but merely received sludge wasted from MBR 1. The metabolism of the microbial population in MBR 2 was proposed to be different to that of MBR 1, i.e. in a state of lysing / endogenous respiration and starvation rather than growing as in MBR 1. This is being discussed in more detail elsewhere in the thesis (refer to Chapter 2).

Assuming that lysis and cryptic growth was occurring in MBR 2, sludge from MBR 2 was sonicated, centrifuged and the supernatant was filtered to obtain the lysis product as a substrate required for the batch respirometric kinetic studies. However, as it can be seen in Figure 5.5, there was no obvious growth to be observed and it was not possible to determine the growth kinetic parameters such as μ_m and K_s. The comparison of Figure 5.5 with Figure 5.1 shows a dramatic difference between the tests carried out for MBR 1 and MBR 2 and further confirms that there is very little growth in MBR 2. The experiment was repeated with a higher F/M ratio range (0.033 to 1.101) but it was still not possible to determine the growth kinetics.

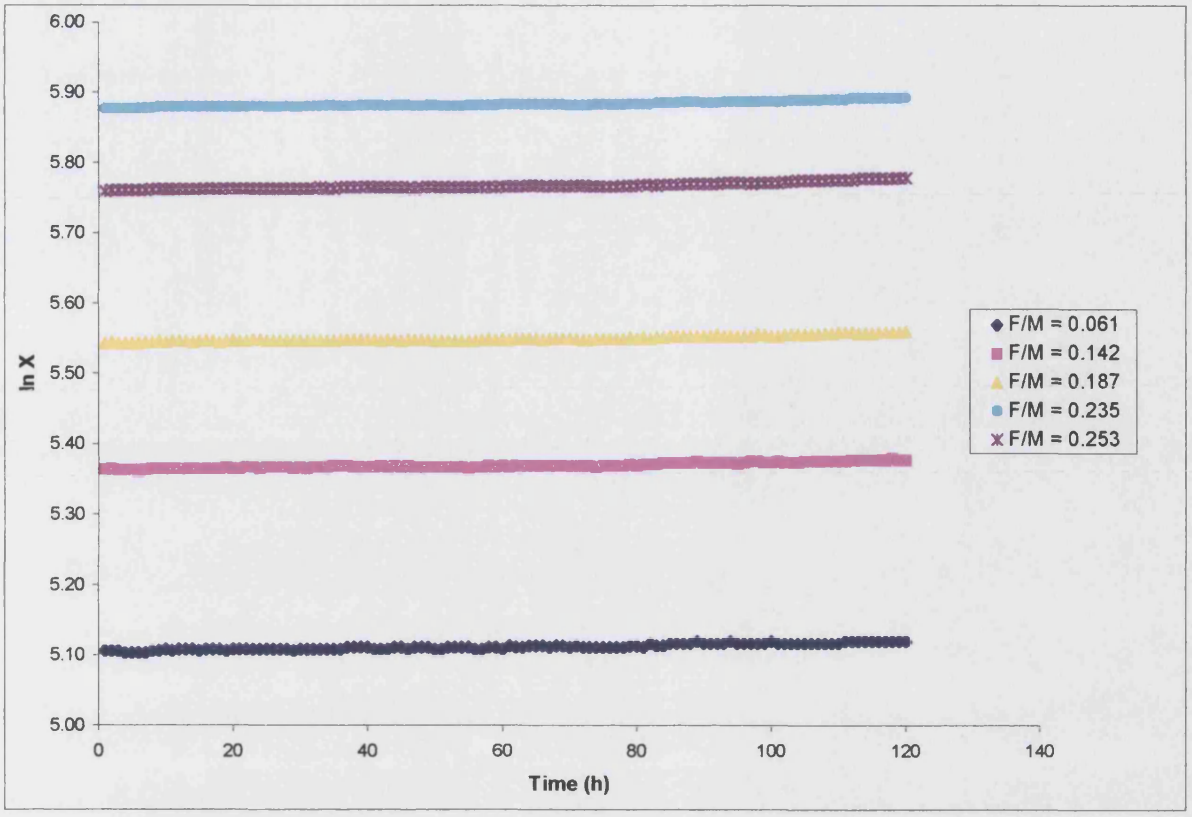


Figure 5.5: Ln biomass (X) growth curve against time for MBR 2 with F/M ratio between 0.061 and 0.253

Permeate discharged from MBR 1 was also used as a substrate for the inoculum from MBR 2 but it was unsuccessful. The last attempt was carried out with sonicated sludge from MBR 1 and its lysis product was used as a substrate. Interestingly, with the F/M ratio ranging between 0.469 to 1.222, the following kinetic parameters were obtained from the respirometric batch studies. Both μ_m and K_s were found to be 0.0033 h^{-1} and 86.03 mg COD/l. However, the results were not repeatable; therefore the validity of the kinetics determined for MBR 2 in this work is under question until further future work can be carried out to verify their validity.

5.5.3 Estimation of k_d for MBR 1

The biomass decay rate, k_d was determined from the batch respirometric studies when the biomass was respiring endogenously without any food supply. Table 5.10 and Figure 5.6 show the results of k_d determined for MBR 1 at different selected time periods.

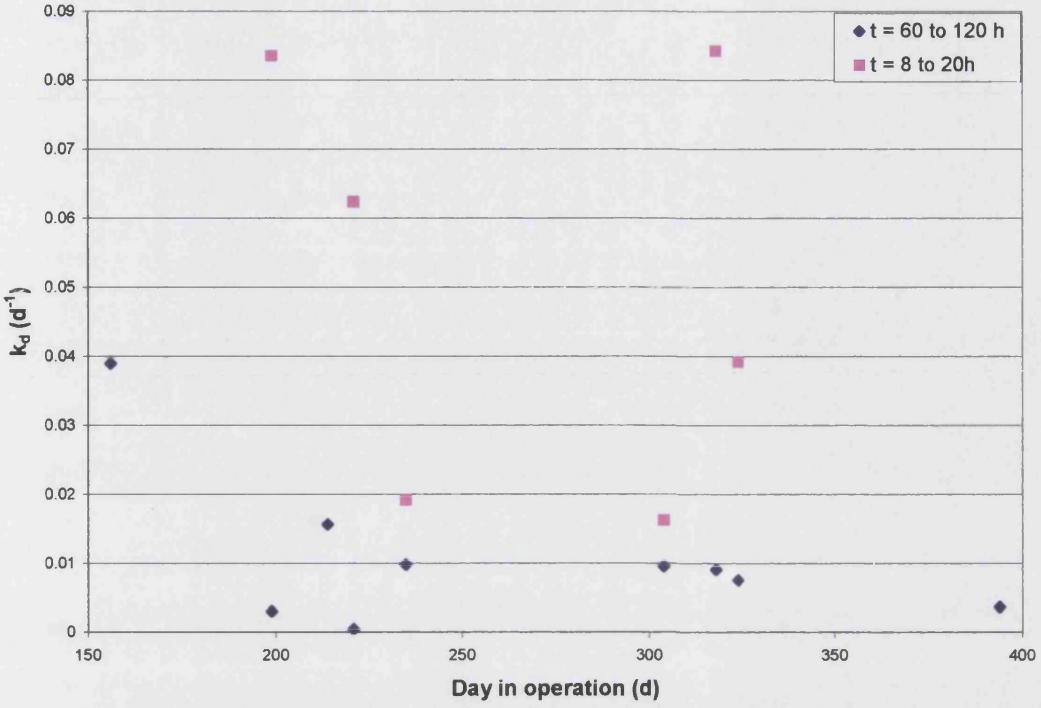


Figure 5.6: k_d of MBR 1 determined at different time periods *

**Note: MBR 1 at steady state, with operating conditions of HRT = 6 h and SRT = 16 d).*

Table 5.10: Experimental k_d of MBR 1 (found from batch respirometry) determined at different time periods. *

Days of operation	k_d taken from t = 60 to 120 h (d^{-1})	k_d taken from t = 8 to 20 h (d^{-1})
156	0.0390^	N/A
199	0.0031	0.0836
214	0.0157	N/A
221	0.0005^	0.0624
235	0.0099	0.0192
304	0.0096	0.0163
318	0.0091	0.0842
324	0.0076	0.0392
394	0.0037	N/A
Average ± std. dev.	0.0084 ± 0.004	0.051 ± 0.03

^ Possible error points excluded from the calculation of the average value

**Note: MBR 1 at steady state, with operating conditions of HRT = 6 h and SRT = 16 d).*

Figure 5.6 shows the comparison of the experimental k_d of MBR 1 determined at different time periods, between 60 to 120 h and between 8 to 20 h. The graph shows great scattering for both sets of k_d. It can be observed that k_d values determined between 8 to 20 h was higher compared to those determined between 60 to 120 h. This could be due to the biomass not respiring endogenously because there is still residual substrate present at the earlier time period. Hence, the k_d determined between 60 to 120 h would be more representative of the real endogenous decay rate. Due to the scattering, it was hard to determine the average k_d. The average of k_d was taken to be 0.0084 ± 0.004 d^{-1}, after excluding the two possible error points found on day 156 and 221. On day 156, the k_d value found was much higher than the rest of the k_d values and this could well be caused by the residual substrate still present in the test sample (sample was not well washed). As for on day 221, the k_d found was very low, possibly due to the very low respiration rate (sample was not well prepared, less sludge inoculum in the test) and the results was not recorded by the OxiTop® system.

The k_d or b_H values reported in the literature, either assumed for mathematical modelling or experimentally determined, shows wide variability (refer to Table 5.3). The b_H values varied between $0.077 - 0.25$ d^{-1} under different conditions such as sludge age, temperature, wastewater characteristics, etc. Metcalf and Eddy (1991) reported k_d for the activated sludge process for domestic wastewater to be between $0.025 - 0.075$ d^{-1}, with a typical value of 0.06 d^{-1}. However, the average k_d for MBR 1 determined between 60 to 120 h (0.01 d^{-1}) is much smaller compared to the reported values. In comparison, the average k_d for MBR 1 determined between 8 to 20 h (0.05 d^{-1}) is more in agreement with the reported values. This presented a dilemma as to which set of results are more realistic, bearing in mind that the results determined between 8 to 20 h could be associated to residual substrate present at the beginning of the test, thus not representing the real endogenous respiration rate. This has to be further investigated and a more refined protocol for determining k_d should be proposed in order to obtain a more comparable and realistic result of k_d for MBR 1.

Avcioglu *et al.* (1998) remarked that the endogenous decay process can be considered as an area with the least amount of experimental support, both in terms of method of assessment and available data readily and reliably usable in modelling. They found a

b_H value of 0.09 d^{-1} from their work, which is also markedly lower than the reported values in the literature (typically 0.24 d^{-1} at 20 °C). They commented that the difference is partly attributed to the variations in wastewater characteristics but mainly to the limitations of the currently available respirometric procedure. The procedure suffers from the analytical limitations associated with O_2 measurements in the course of the experiment with gradually decreasing biomass activity. Therefore, future advances in the development of a more accurate and sensitive equipment as well as a more refined and comparable respirometric protocol would be beneficial for the determination of the kinetic parameters.

5.5.4 k_d for MBR 2

The results of k_d obtained for MBR 2 are summarised in Table 5.11 and Figure 5.8. If the assumption of endogenous respiration / lysis being the more dominant phenomenon in MBR 2 was valid, it is possible to calculate the decay coefficient from the steady state mass balance model performed around MBR 2 (see Figure 5.7), which is given by Equation 5.14.

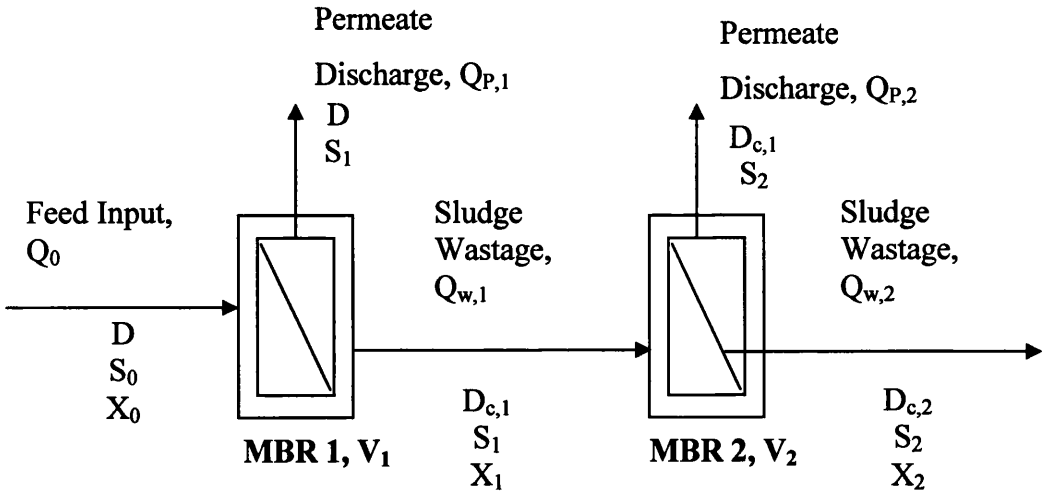


Figure 5.7: Schematic diagram of a two-stage MBR system

From the following equation, the decay coefficients were calculated, and then compared with the experimental findings. The results are tabulated in Table 5.11.

$$Q_{w,1} X_1 - Q_{w,2} X_2 = k_{d,2} X_2 V_2 \qquad (5.14)$$

where $Q_{w,1}$ = Sludge wastage rate into MBR 2 from MBR 1, l/d

X1 = MLSS concentration of MBR 1 under steady state condition, g/l

$Q_{w,2}$ = Sludge wastage rate from MBR 2, l/d

X_2 = MLSS concentration of MBR 2 under steady state condition, g/l

$k_{d,2}$ = biomass decay coefficient of MBR 2, d^{-1}

V_2 = working volume of MBR 2, l

Table 5.11: Theoretical biomass decay coefficients, k_d (d^{-1}) calculated with Equation 5.14 and experimentally determined k_d from the batch respirometric studies for MBR 2. *

Days of operation	Theoretical k_d (d^{-1})	Experimental k_d (d^{-1})
235	0.0136	0.0640
242	0.0158	0.0154
263	0.0155	0.0057
269	0.0142	0.0264
283	0.0153	0.0099
297	0.0138	0.0043
Average ± std. dev.	0.0147 ± 0.0009	0.0209 ± 0.023

**Note: MBR 2 at steady state, with operating conditions of HRT = 19 d and SRT = 100 d).*

As seen from Figure 5.8, the theoretical calculated k_d showed consistent results with an average value of 0.0147 ± 0.0009 d^{-1}. On the other hand, the experimental k_d showed very scattered results. As it can be seen from Table 5.11 and Figure 5.7, the comparison of theoretical and experimental obtained k_d also showed great deviations from one another. Equation 5.14 defined the endogenous respiration coefficient as a

lumped together-simplified value representing a decrease in cell mass (Metcalf and Eddy, 1991), incorporating many different mechanisms (e.g. maintenance, cell lysis, cryptic growth, predation, etc.) that are still not well understood. Readers are referred to Chapter 2, for a short review of the different metabolic processes. Therefore, the results obtained from this assumption might carry some uncertainties in its accuracy. Until we can fully understand the different mechanism happening in real life, there is no completely right or wrong answer.

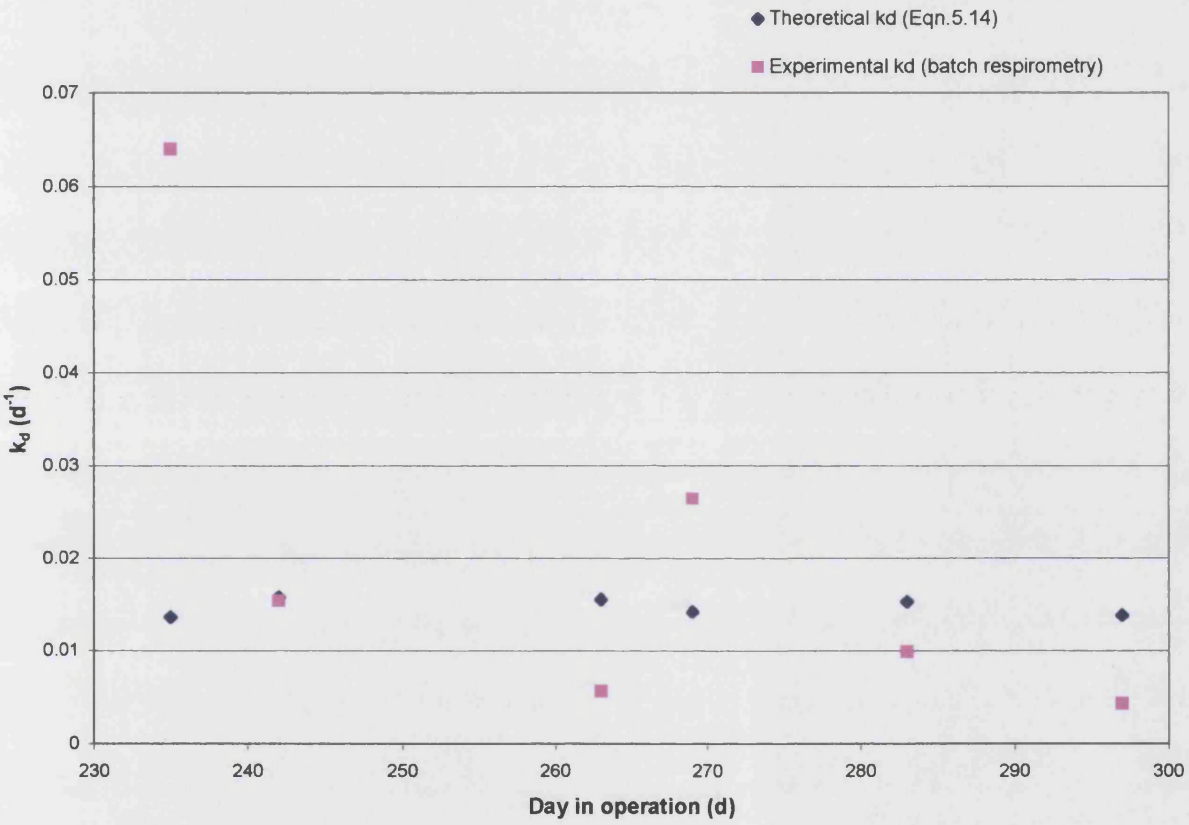


Figure 5.8: Comparison of theoretical (calculated with Equation 5.14) and experimental (found from batch respirometry) k_d of MBR 2

The accuracy of the experimental findings could be questioned as well. The scattering of the experimental results could have been due to several factors including the sensitivity of the oxygen sensor, the stability of the respirometry batch system and the consistency of experimental procedures carried out. The interpretation of the results was made difficult in terms of deducing the time period where the biomass was in the phase of endogenous respiration. In this case, the time period chosen was well

towards the end of the experiments (t = 60 to 120 h in a 5-day test) where any possible residual substrate carried over (either in the dilution water or in the biomass not being washed properly) might have been exhausted. In addition, there were no standardised experimental procedures in the literature for the determination of k_d and therefore the results published from the literature could be very subjective and case sensitive (different for each case as the activated sludge population is very complex). In conclusion, further understanding of the kinetics in a microbial level will assist us in developing and refining the procedure for kinetics determination.

The experimental k_d determinations were also made difficult by the fact that the activated sludge population is a very dynamic system because the microbial population is experiencing changes constantly and this might have an effect on the k_d determined over the different period of time. Due to limitations of equipment, replicates were not allowed as kinetics parameters such as μ_m and K_s were also being determined in the same batch of experiments.

5.6 Conclusions

In the recent years, there has been a rapid development and a huge leap in the mathematical modelling of activated sludge. However, there exists a gap between the modelling and experimental support because the experimental backup in the determination of the model coefficients has lagged behind. Therefore, the aim for future studies should be focused on developing more reliable and rapid methods for kinetic and stoichiometric parameter determination. In addition to that, the establishment of a more standardised and refined protocol for batch respirometric studies will ensure better consistency and comparability of the kinetic parameters to be determined by different researchers in the future. Moreover, close collaboration between the different expertise in the area of microbiology and engineering is essential towards the progress of mathematical modelling or understanding of the activated sludge processes.

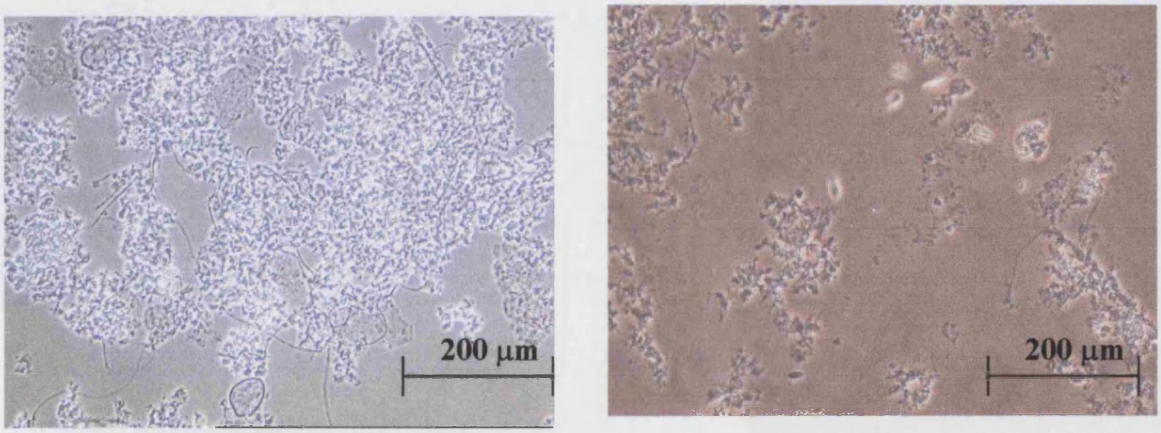
Chapter 6: Microscopic Observations

Scope of Chapter 6

In this chapter, microscopic pictures of the cultures in MBR 1, the original MBR 2 and the new MBR 2 are shown. Higher organisms such as cillates, rotifers and nematodes were observed in all the MBRs. Ex-situ experiments to achieve sludge reduction by predation of higher organisms were carried out with *Acanthyst* amoeba on sludge from the original MBR 2.

6.1 MBR 1

With the help of an expert microbiologist, the following description was given of MBR 1. Confluent mass of very small cells, with no flocs, was observed. Ciliated and stalked protozoa, rotifer and nematode were observed in MBR 1, as seen from Figures 6.2 – 6.5. As MBR 1 has a more viable bacterial population, the higher organisms present in MBR 1 are more abundant than that in both MBR 2.



(a) (b)

Figure 6.1: Microscopic examination of activated sludge in MBR 1 (a) Picture taken on Day 249 (b) Picture taken on Day 319

6.1.1 Ciliates

Ciliates are single-celled protozoa that can be recognised by their hair-like cilia. They use them to propel themselves, as well as for feeding. The ciliates that were discovered in MBR 1 were suspected to be colpidium. They have flattened kidney-

shaped bodies, with the mouth situated at the base of a short groove located just below a slightly jutting or twisted anterior part of the body. [see Figure 6.2 (a) and (b)]. This ciliate is often found in large numbers in organically enriched and slightly anoxic sites. The cell is evenly ciliated. The body length can be between 40 –100 μm.

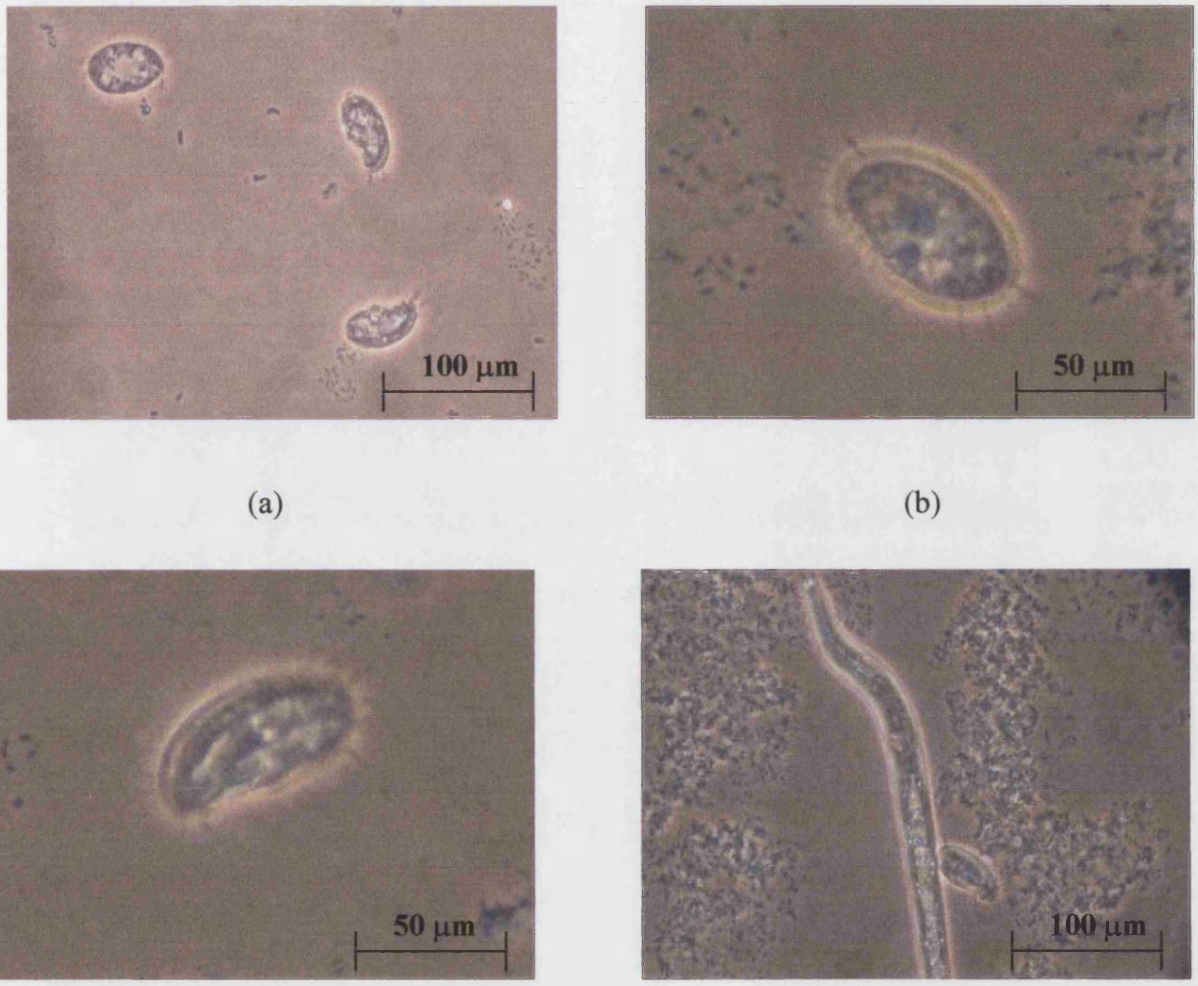
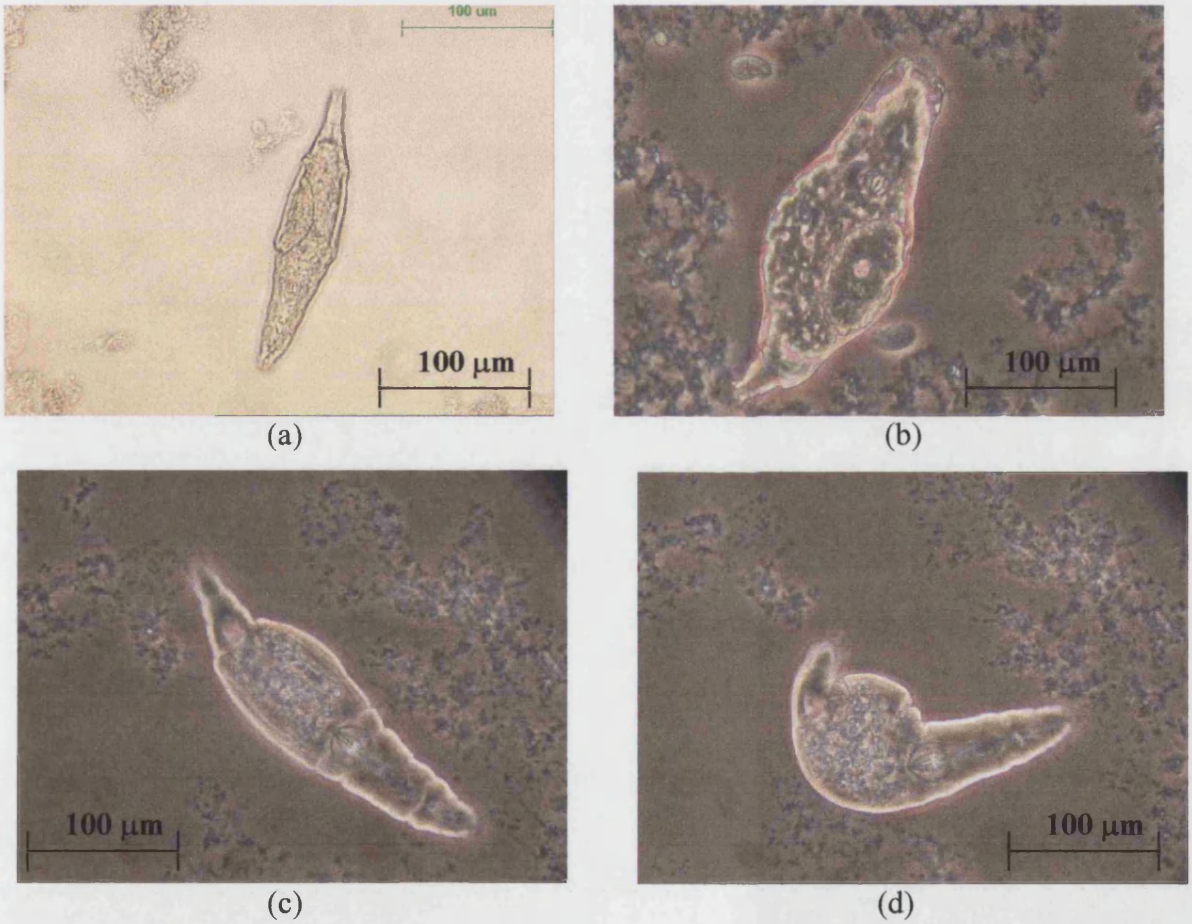


Figure 6.2 (a) – (d): Colpidium found in MBR 1 (d) showing a nematode with colpidum

6.1.2 Rotifers

Rotifers form part of the metazoa group and they are multi-celled. They can measure up to 2 mm in length, though few exceeded 0.5 mm. The name derived from Rota, Latin for wheel and they are recognisable by their single, double or sometimes multiple crown-like corona of cilia which appear to rotate. The species that was found in MBR 1 was identified to be rotaria. The body of rotaria has telescopic segments

and they can expand or retract like a telescope [refer to Figure 6.13 (c) and (d)]. When fully expanded, it can reach up to a length of 200 μm [see Figure 6.3 (a)]. The body is transparent and in their living state, their internal organs are clearly visible. Rotaria uses its ciliated crown for locomotion. They can also creep with its head and tail end. It uses the stickiness of the tail or podite to attach itself to a surface while feeding [as seen from Figure 6.3 (d)]. The feeding is carried out by making a current with the crown of its cilia to sweep in food [see Figure 6.14 (a) for a clearer picture of feeding.]. The food is then passed into the gut via two stout grinding plates called the mastax.



(a) (b)

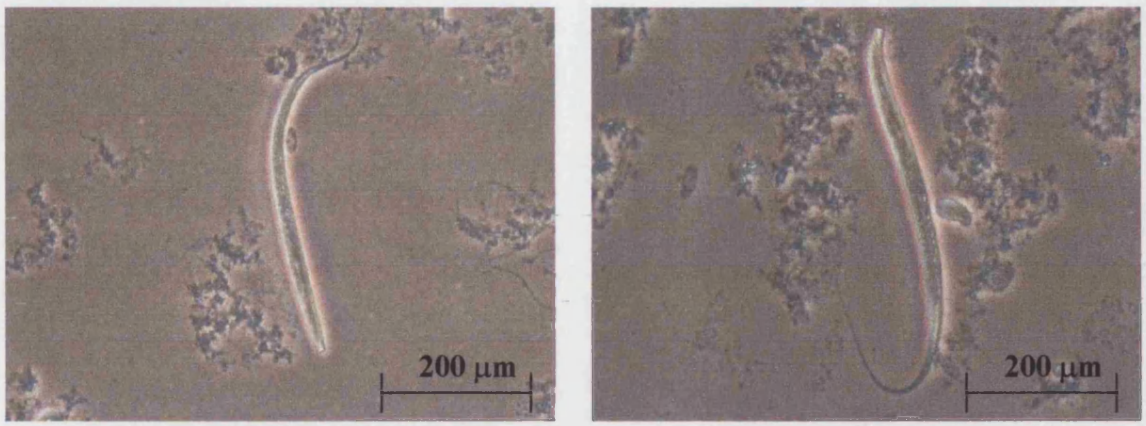
(c) (d)

Figure 6.3 (a) – (d): Rotaria found in MBR 1

6.1.3 Nematodes

Nematodes were also found in MBR 1. They are extremely common and widespread metazoa. Most nematodes have a long thin shape and a slightly blunt anterior end. They are rather stiff and move either by writhing (lashing) or by gliding through the

substrate. At higher magnifications, a strong muscular pharynx[1] can be seen near the front, and egg-bearing ovaries near the back end [refer to Figure 6.17 (e) and (f)].



(a) (b)

Figure 6.4 (a) and (b): Nematode found in MBR 1

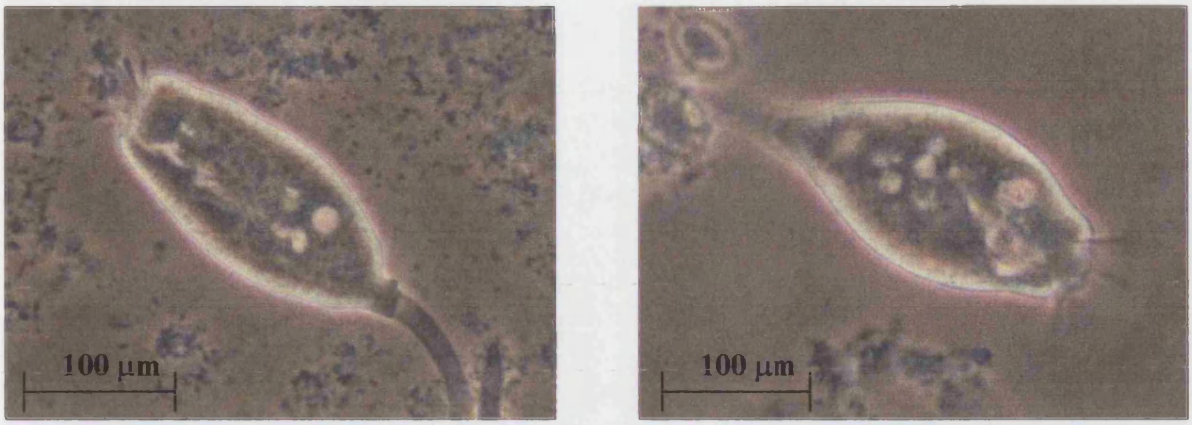
6.1.4 Vorticellae

A large quantity of vorticellae was found in MBR 1. It is a peritrich[2] ciliate. The cilia present are the wreaths of feeding cilia made of an upper band, which corresponds to a membranelle and generates currents of water for feeding; and a lower band, which corresponds to the undulating membrane. Food is passed through a buccal[3] cavity before being packaged within food vacuoles [see Figure 6.5 (a) and (b)]. Many peritrichs are supported on stalks, which makes them vulnerable to predators (Patterson, 1996). Some protection is provided by stalk contractility, afforded by a spirally contractile thread or spasmoneme[4]. The contractile elements of the spasmoneme pass into the body.

The length of cell varies from species to species; most are between 20 and 200 μm, most commonly 40 – 80 μm. It is a very common genus with a bell-shaped body. Vorticella was found to be in single stalk, groups of two or three connected on the stalk or a big colony [see Figure 6.6 (a) – (d)].

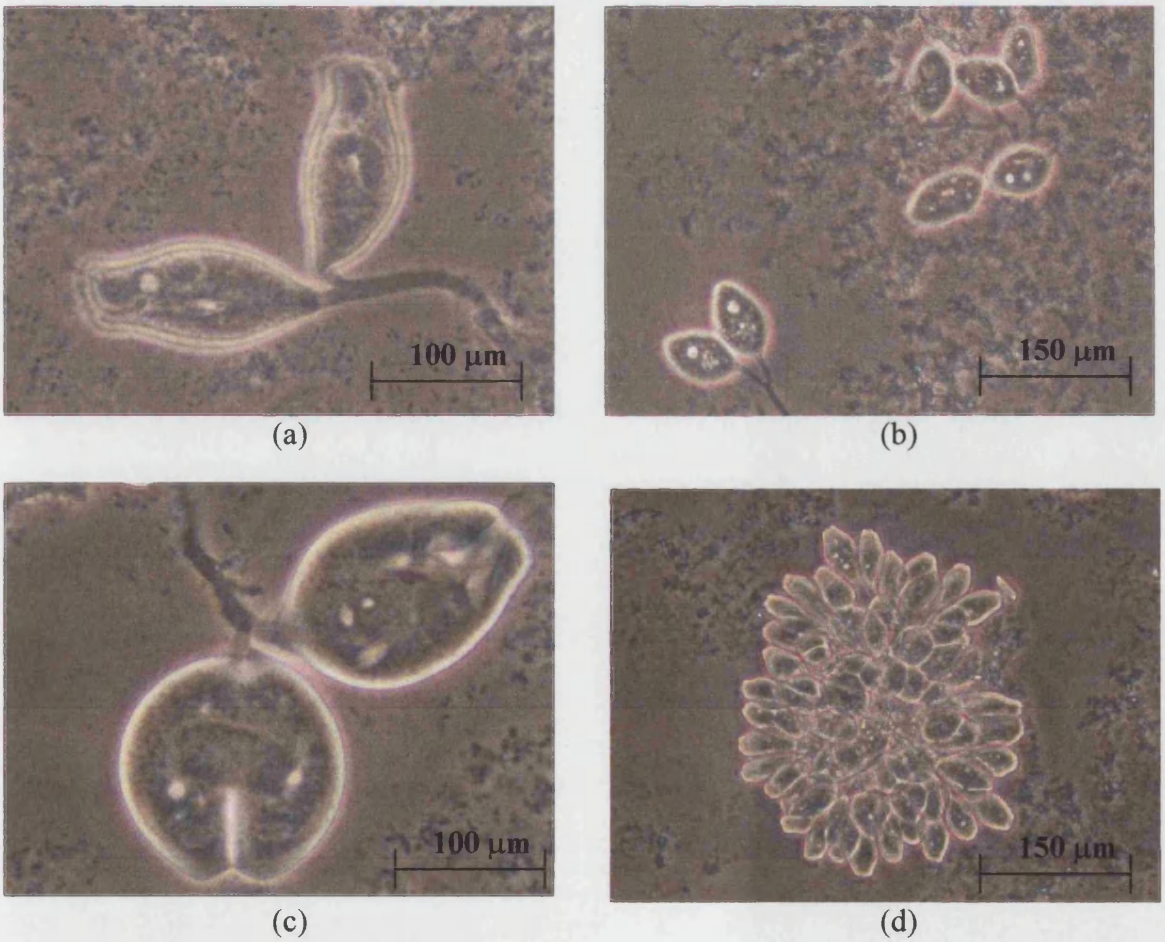
Glossary of terms (Patterson, 1996)

1. Pharynx: a region of the ingestion apparatus that lies internal to the mouth of a metazoan organism, involved in the swallowing process.
2. Peritrich: a type of oligohymenophoran ciliate in which one membranelle and the undulating membrane are greatly lengthened, spiralling around the oral end of the body.
3. Buccal: relating to mouth structures (e.g. buccal ciliature).
4. Spasmoneme: a contractile element in the stalk of some peritrich ciliates.



(a) (b)

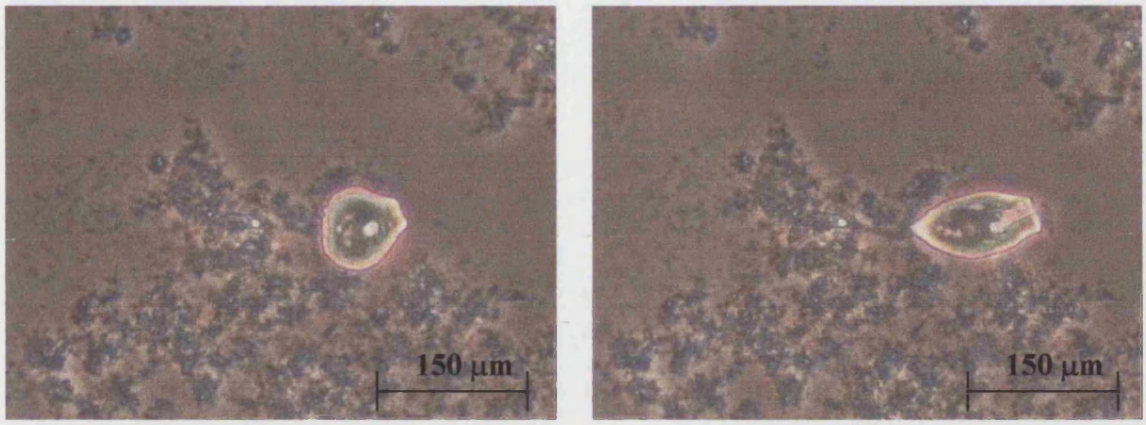
Figure 6.5 (a) and (b): **Single stalked bell-shaped vorticella. Buccal cavity near the oral end can be seen. Food vacuoles and macronucleus present in the cell are visible**



(a) (b)

(c) (d)

Figure 6.6: **Vorticella growing in colonies: (a), (b) and (c) showing them commonly found in groups of two or three (d) shows a large round colony**



(a) (b)

Figure 6.7: (a) showing a contracting vorticella into a round body (b) showing itself released back into a normal bell-shaped body

Figure 6.8 (a) – (d) shows vorticella dividing. Like many sessile[5] protozoa, vorticella produces a motile daughter cell. It is called a swarmer[6] or telotroch larva. The daughter cell develops near the base of the parental cells. The daughter cell has an additional wreath of cilia, the trochal band, which is used for propulsion. The basal trochal cilia are lost after the larva has found somewhere to settle [see Figure 6.9 (c) and (d)]. Trophic[7] cells may also produce a basal band of cilia under unfavourable conditions.

Glossary of terms (Patterson, 1996)

5. Sessile: refers to organisms that are fixed to the substrate, by e.g. a stalk or lorica.
6. Swarmer: a stage in the life cycle of some protists. Its function is distributive, i.e. it moves away from the location of the parent cell, potentially to colonize other sites. Occurs mostly as the motile stage of sessile organisms, such as peritrich ciliates or suctorian ciliates.
7. Trophic: said of organisms that are active and feeding. Contrasts with the encysted state, theronts and swarmers. May also be used to refer to those aspects of metabolism associated with growth.

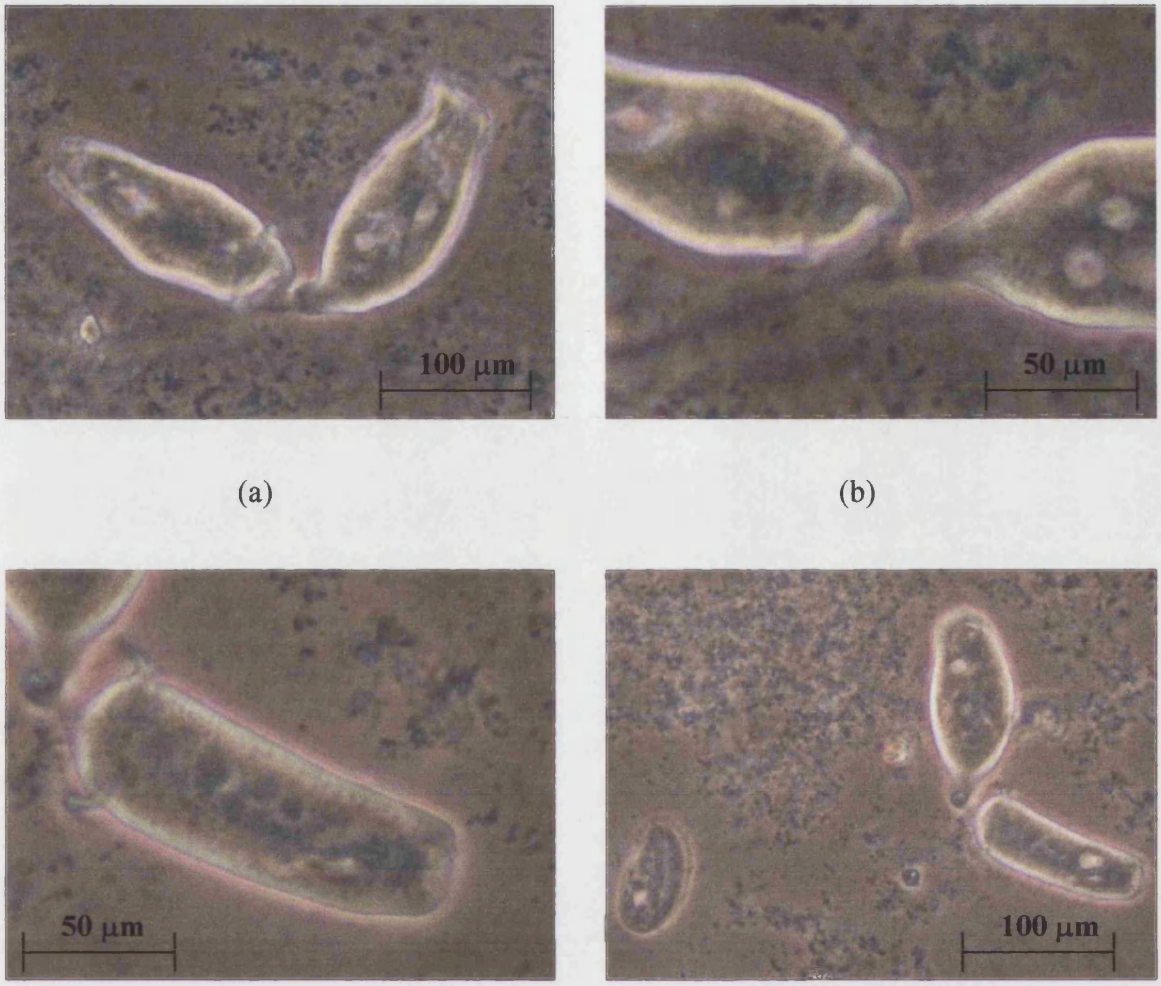


Figure 6.8 (a) – (d) showing vorticella dividing and the basal trochal cilia is visible of the daughter cell

Figure 6.9 (a) and (b) shows a peritrich telotroch larva. It is a motile swarmer produced by cell division. The cell swims around until it finds a suitable place to settle. Propulsion occurs by means of a basal wreath of cilia. The anterior end of the cell is pulled in, so that no feeding occurs. Consequently, no food vacuoles are present in the cell, but the buccal cavity and a part of the macronucleus are visible. Figure 6.9 (c) showed a single loose cell body, which will develop into a full grown vorticella supported by a stalk [as seen in Figure 6.9 (d)].

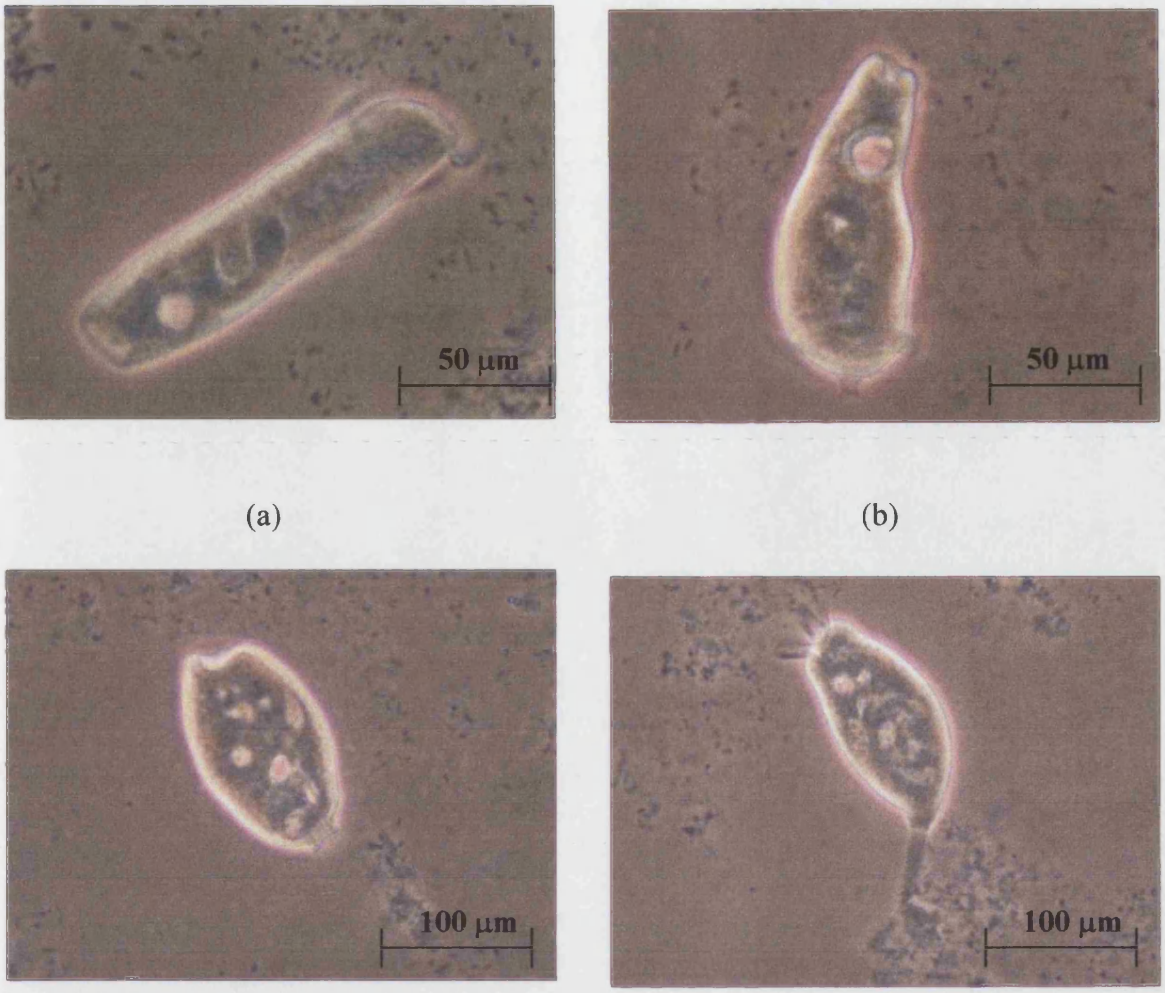
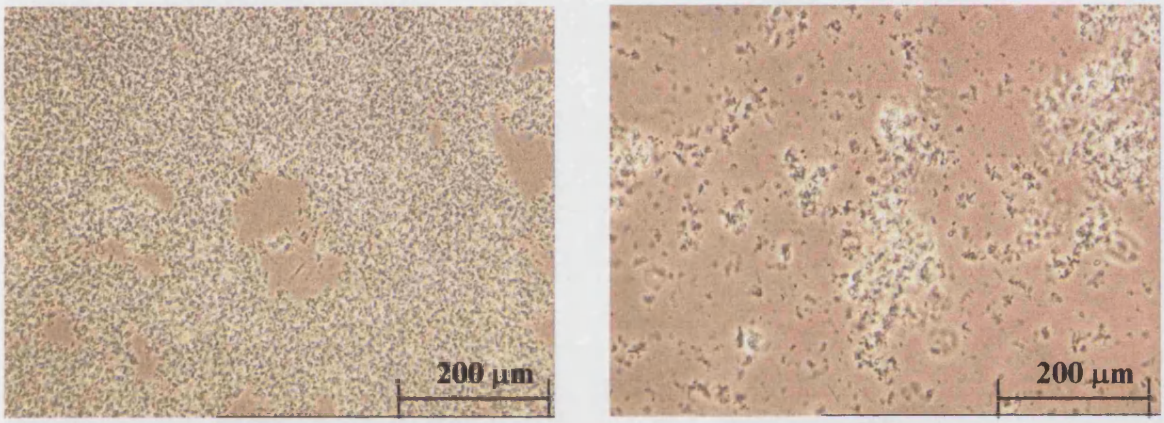


Figure 6.9: (a) and (b) showing a motile larva swimming rapidly around (c) and (d) showing the single cell eventually finding a place and grown into a stalked vorticella

MBR 1 has a more viable bacterial population as evidenced from the higher biological activity measured as "specific oxygen uptake rate" (SOUR) (see Section 4.3). The higher bacterial viability is due to MBR 1 operating at a smaller SRT and a high organic loading rate, to promote bacterial growth and the degradation of organic waste in the synthetic feed. As a result, the higher organisms were found in abundance as they fed on bacteria in MBR 1. It could be seen also from the microscopic observations that the protozoan population is not as diverse as it would be expected in real sewage treatment works, as synthetic sewage is employed in this work.

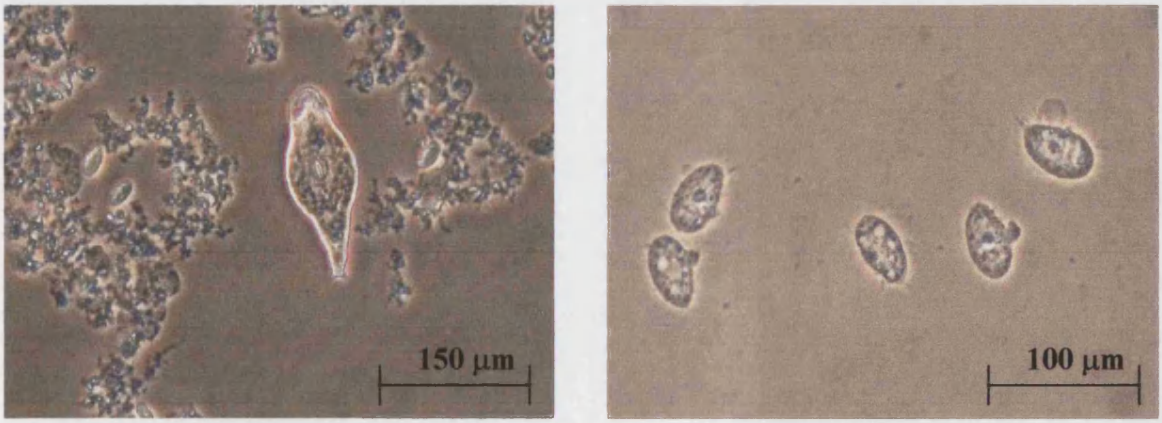
6.2 Original MBR 2

Figure 6.10 (a) and (b) shows the microscopic examination of the activated sludge in the original MBR 2. Confluent mass of very small cells and no floc was observed. The observations made on Day 249 show no sign of protozoa or other higher organisms. However, the pictures taken on Day 160 clearly showed the existence of higher organisms in the system [see Figure 6.11 (a) and (b)]. The extinction of the protozoa and metazoa was thought to be due to the build up of acid / gradual pH drop in MBR 2 over with time, as a result of nitrification (see Section 4.5.4). The bacterial population is also less viable as endogenous metabolism dominates in MBR 2.



(a) (b)

Figure 6.10: Dense structure of the activated sludge in the original MBR 2, picture taken on Day 249 (a) No dilution (b) 10 % dilution



(a) (b)

Figure 6.11: (a) showing the existence of rotaria and colpidum (b) A large number of colpidium were found in the original MBR 2 (pictures taken on Day 160)

6.3 New MBR 2

The new MBR 2 was started up on Day 309, by receiving sludge from MBR 1. More open cell mass was observed (Figure 6.12). Rotaria, nematode, colpidium and vorticella were observed in the new MBR 2 (Figures 6.13-18). The microscopic description was as follows: approximately 1 rotifer per μL and 1 vorticella per 10 μL.

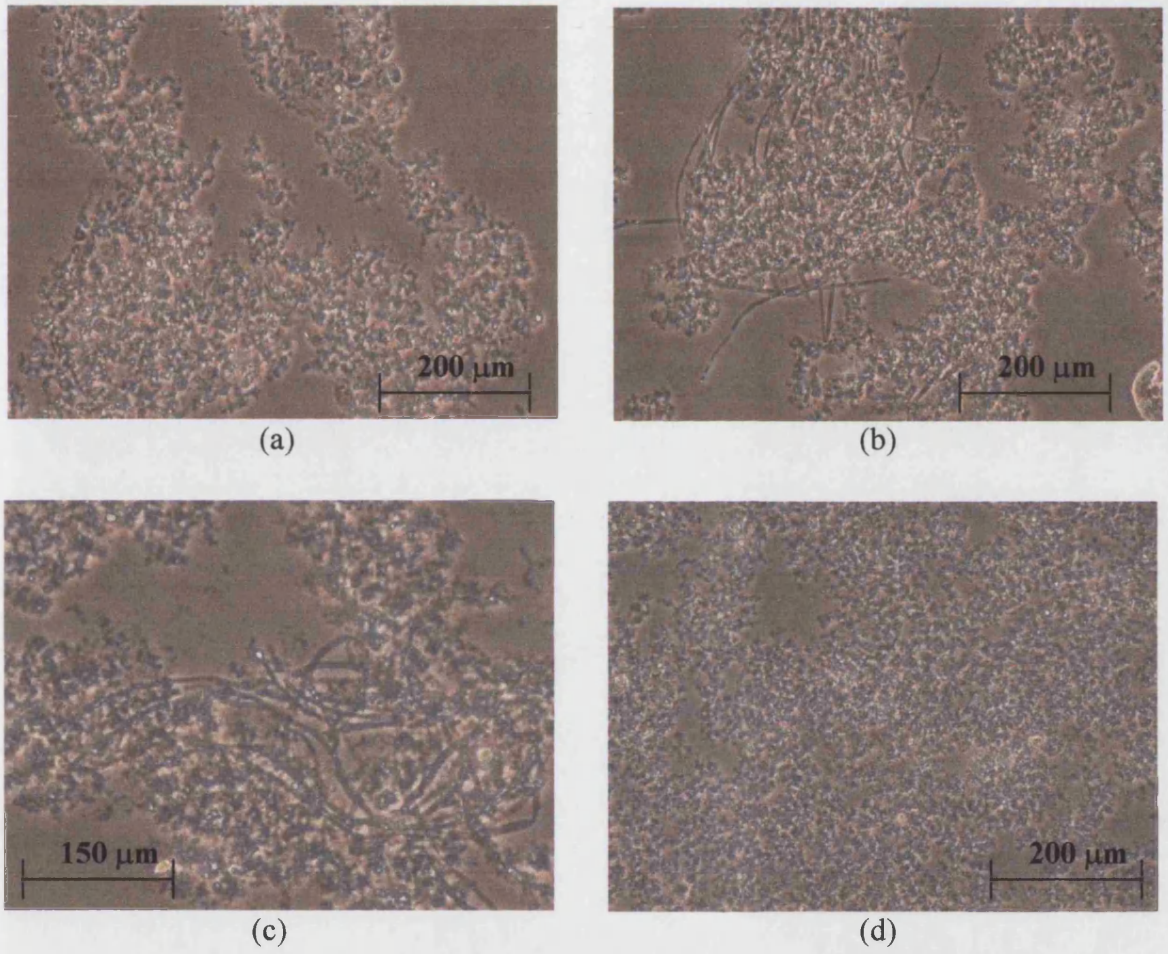


Figure 6.12: Microscopic examination of activated sludge in the new MBR 2. (a) Picture taken on Day 319 (b) and (c) showing filaments (d) Much denser structure, picture taken on Day 466

A healthy and diverse protozoan population was observed in the new MBR 2, even though like the original MBR 2, it also experienced a gradual pH drop due to nitrification (see Section 4.4.1). This could be explained by the protozoan population gradually adapting to the acidic environment. Among the protozoan population, rotaria was present in large quantities. This might be due to the long sludge retention time (100 d), which favours the growth of higher organisms.

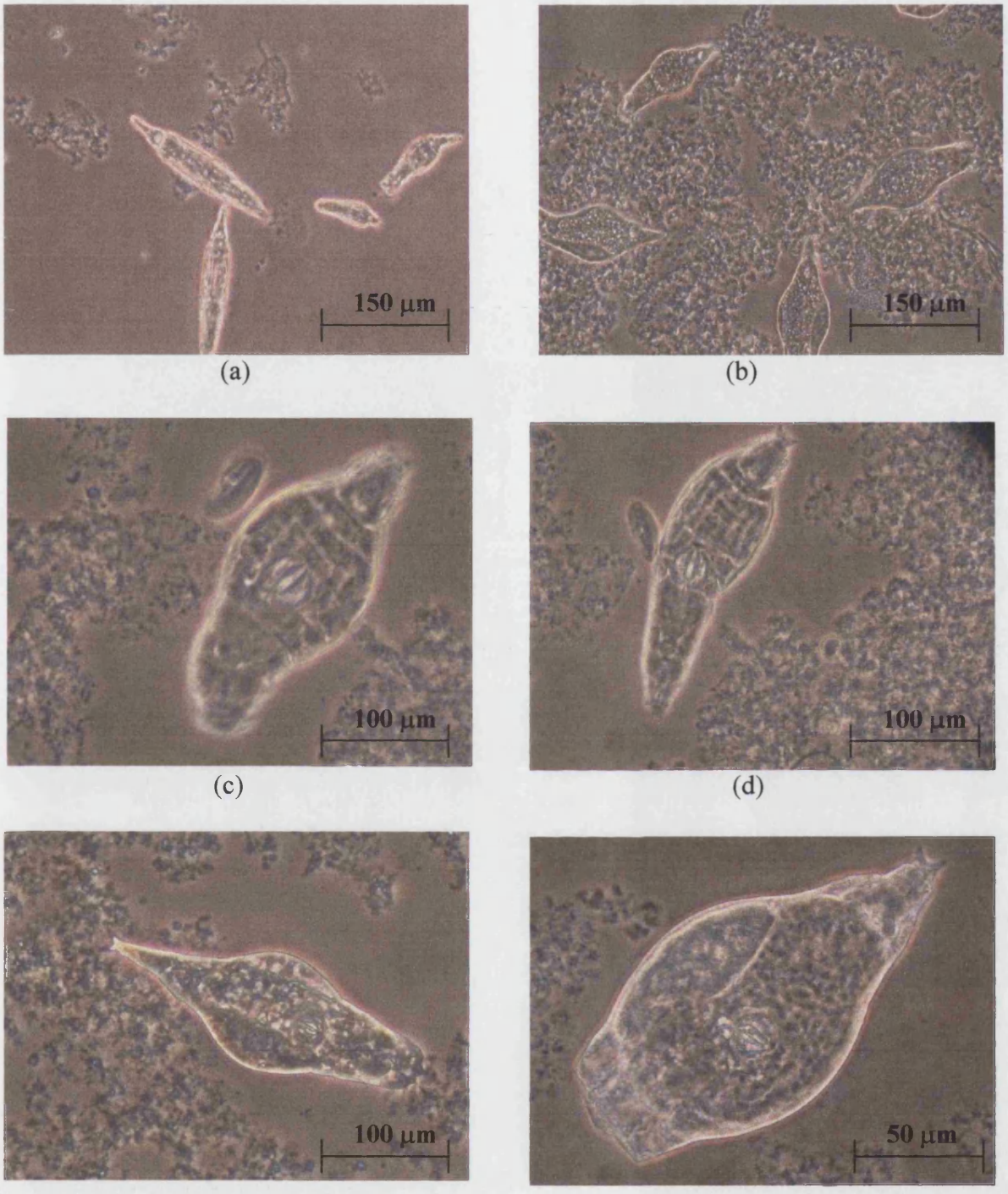
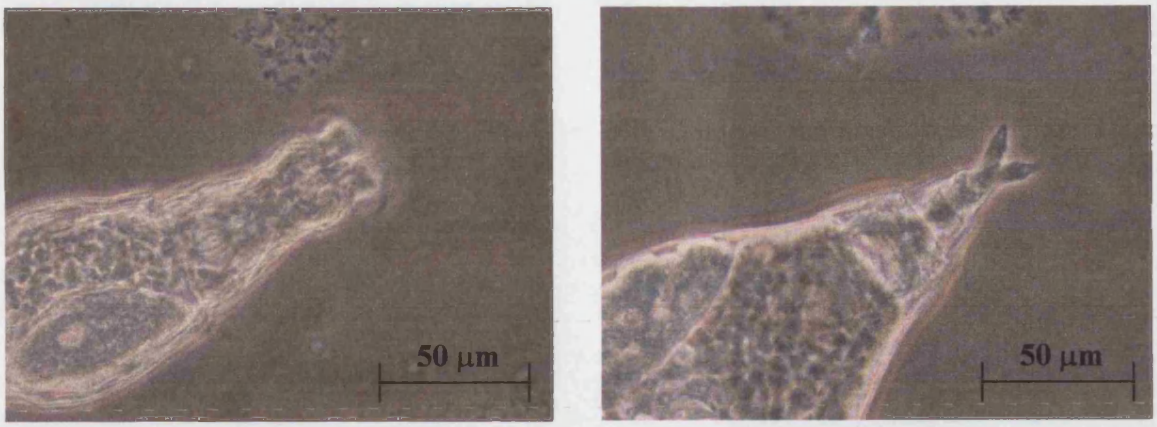
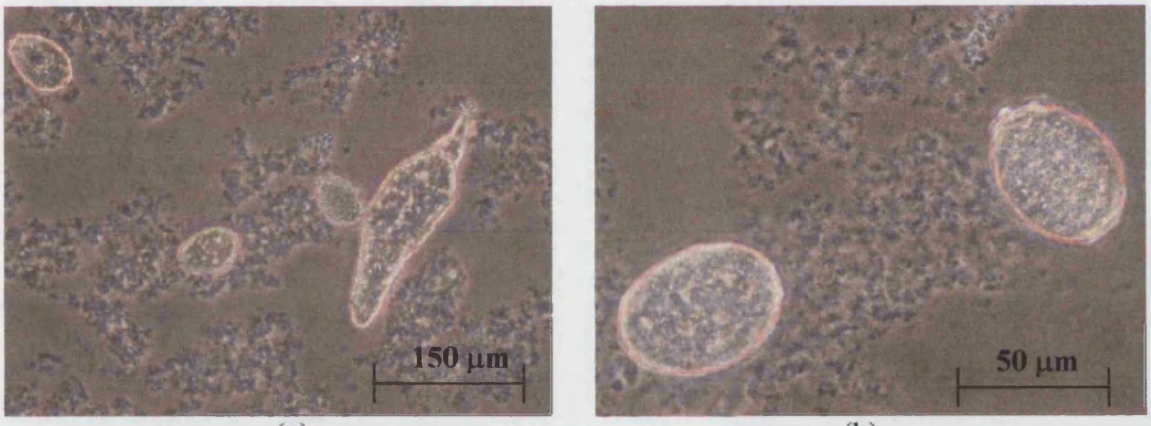


Figure 6.13: **(a) and (b) showing many rotaria (c) and (d) showing the contracted and elongated body of rotaria (d) and (e) showing internal organs of the rotaria**



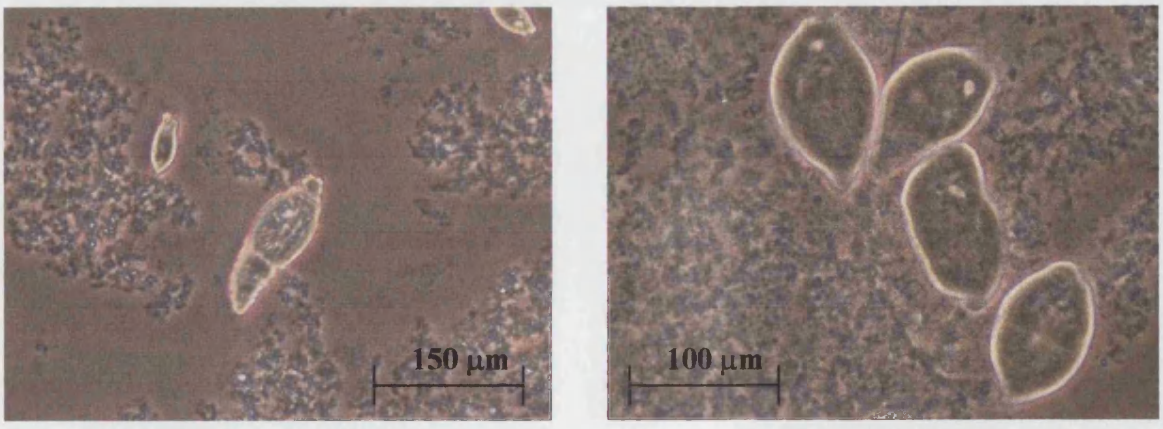
(a) (b)

Figure 6.14: (a) showing the head and the corona cilia used to create a vortex for feeding (b) showing the tail / adhesive podite



(a) (b)

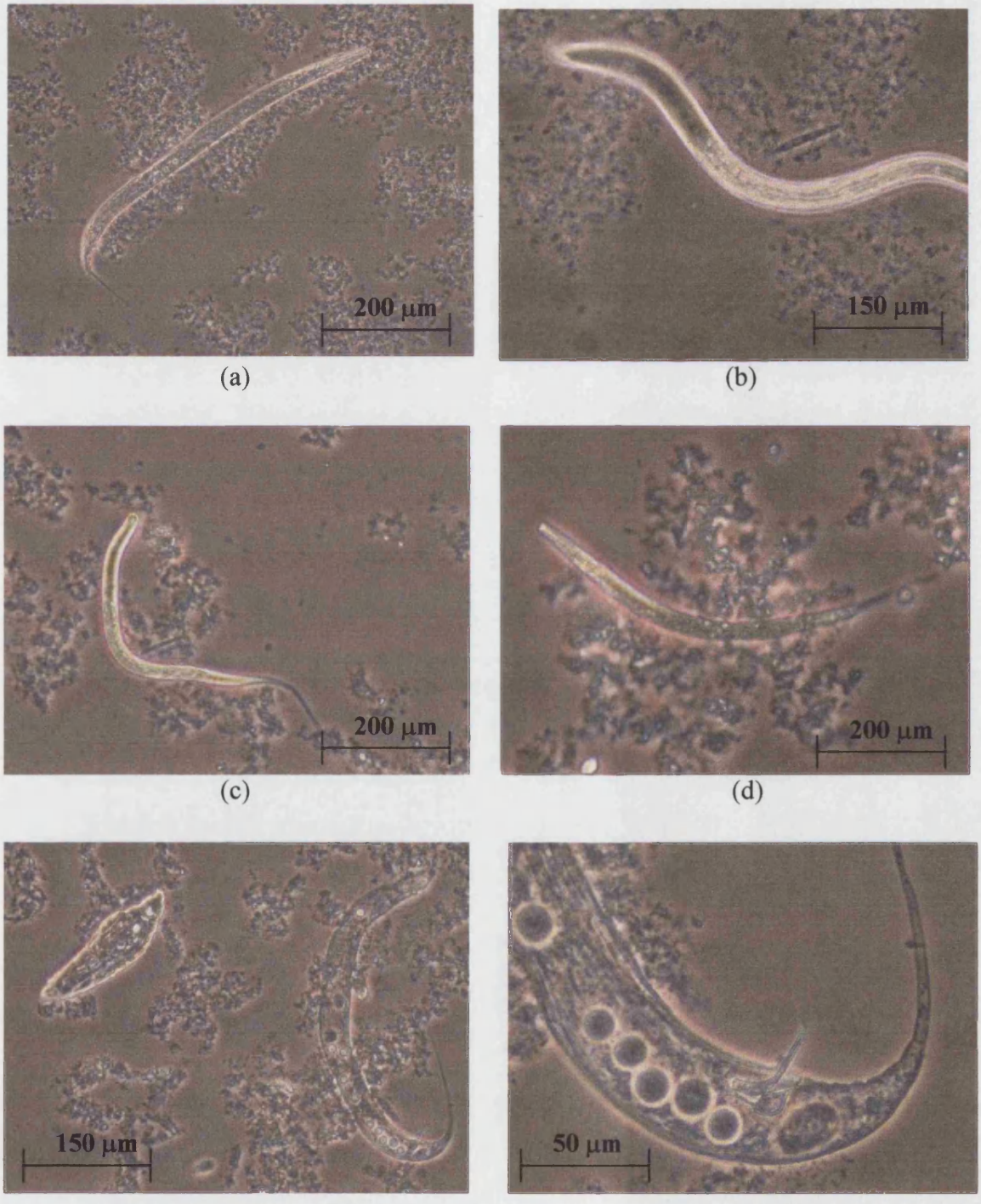
Figure 6.15 (a) and (b): Rotaria form spores under unfavourable conditions



(a) (b)

Figure 6.16(a) and (b): Vorticella found in the new MBR 2 from Day 366

Nematode was also discovered in the new MBR 2. Figure 6.17 (f) showed a clear picture of the ovaries near the back end of the nematode.



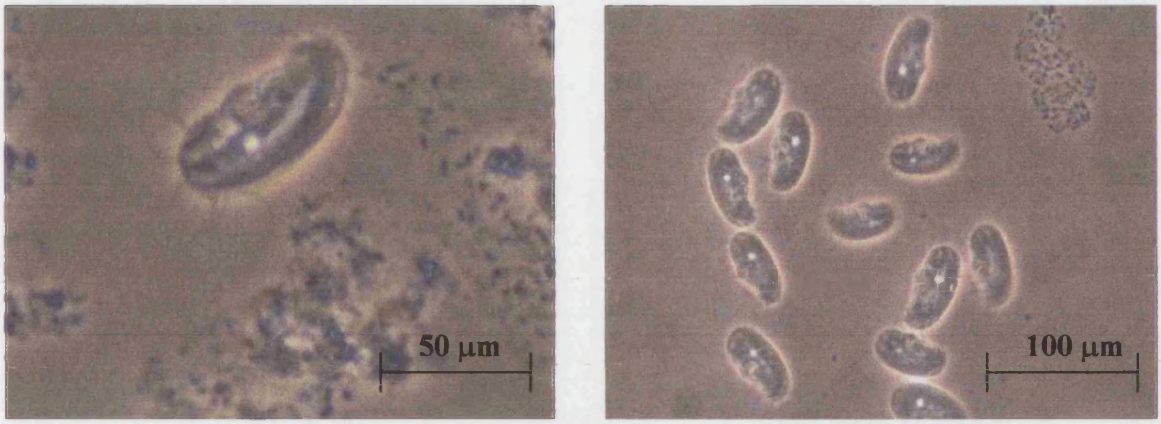
(a) (b)

(c) (d)

(e) (f)

Figure 6.17 (a) – (f): Nematode discovered in the new MBR 2 (f) showing clearly the ovaries at the back end of the nematode

Figure 6.18 (b) shows the many colpidium found in the new MBR 2.



(a) (b)

Figure 6.18: (a) Single colpidium (b) Many colpidium found in the new MBR 2

6.4 Ex-situ experiments with *Acanthyst* amoeba

The manipulation of higher organism in the activated sludge process with the aim of achieving a reduced sludge production had been investigated and reported in the literature (refer to Chapter 2). In this section, the results from ex-situ experiments to investigate the addition of *Acanthyst* amoeba on the sludge from MBR 2 are outlined. *Acanthyst* amoeba was chosen due to its readily availability (Source: Department of Pharmacy, University of Bath, that was also carrying out research with the amoeba).

6.4.1 First set of experiments

6.4.1.1 Materials and Methods

Two 30 ml flasks with water jackets were used for the test. Each contained 20 ml sludge sample withdrawn from MBR 2. At the start, the following were added to each of the two flasks:

A – 2 ml of 10^5 cells/ml amoeba is added

B – 2 ml of saline is added

The water jackets were recirculated with water from a water bath kept at a constant temperature of 20 °C. Slight air bubbling with a fine air stone will provide the oxygen needed for the sludge. Everyday 0.1 ml of sample was taken out of every flask with a pipette and spreaded onto an agar plate. The plates were left overnight in an incubator

kept at 25 °C. On the next day, the agar plates were counted to examine the bacteria number in terms of colony forming unit (CFU) per ml of sample.

6.4.1.2 Results and Discussion

The results are tabulated in Table 6.1 and plotted in Figure 6.19. It can be seen from Figure 6.19 that the bacteria number for both A and B are similar at the beginning of the test (~ 5.4×10^6 CFU/ml). On the following day, there was a 28% reduction in the CFU compared to the initial CFU on day 1 for both A and B. Since then, there was only a slight daily reduction in the bacteria number for flask A (with amoeba) until day 9 (except on day 7, a 0.1% growth can be observed). This may be due to the encysting of amoeba under unfavourable conditions. In contrast, a growth of 23 – 48 % can be observed in the control flask B (without amoeba) for the subsequent 7 days.

Table 6.1: Results for the 1st amoeba test.

Day	Bacteria number (CFU/ml)		% CFU reduction+		Comparison of A and B (% reduction)
	A (amoeba)	B (control)	A (amoeba)	B (control)	
1	5.35 E+06	5.41 E+06	-	-	1.11
2	3.85 E+06	3.93 E+06	28.04	27.36	2.04
4	3.53 E+06	6.65 E+06	34.02	-22.92*	46.92
7	3.55 E+06	7.50 E+06	33.64	-38.63*	52.67
9	3.31 E+06	8.01 E+06	38.13	-48.06*	58.68
14	1.83 E+06	3.32 E+06	65.79	38.63	44.88
16	9.10 E+05	3.39 E+06	82.99	37.34	73.16

*Note: * negative sign (-) indicates growth*

+ % reduction in CFU compared to the initial CFU on day 1

On day 9, another 2 ml (10^5 cells/ml) of amoeba was dosed to flask A. This further led to a reduction in the bacteria number. On day 14, a 66% decrease in CFU was observed and on day 16, 83% decrease could be seen. As for the control flask B, a reduction of 39% in CFU on day 14 and 37% on day 16 was observed.

The comparison of the two systems on a daily basis showed a clear overall reduction in the bacteria number from 1 % (Day 1) to 73 % (Day 14). This demonstrated that the addition of amoeba led to a reduction in the sludge production.

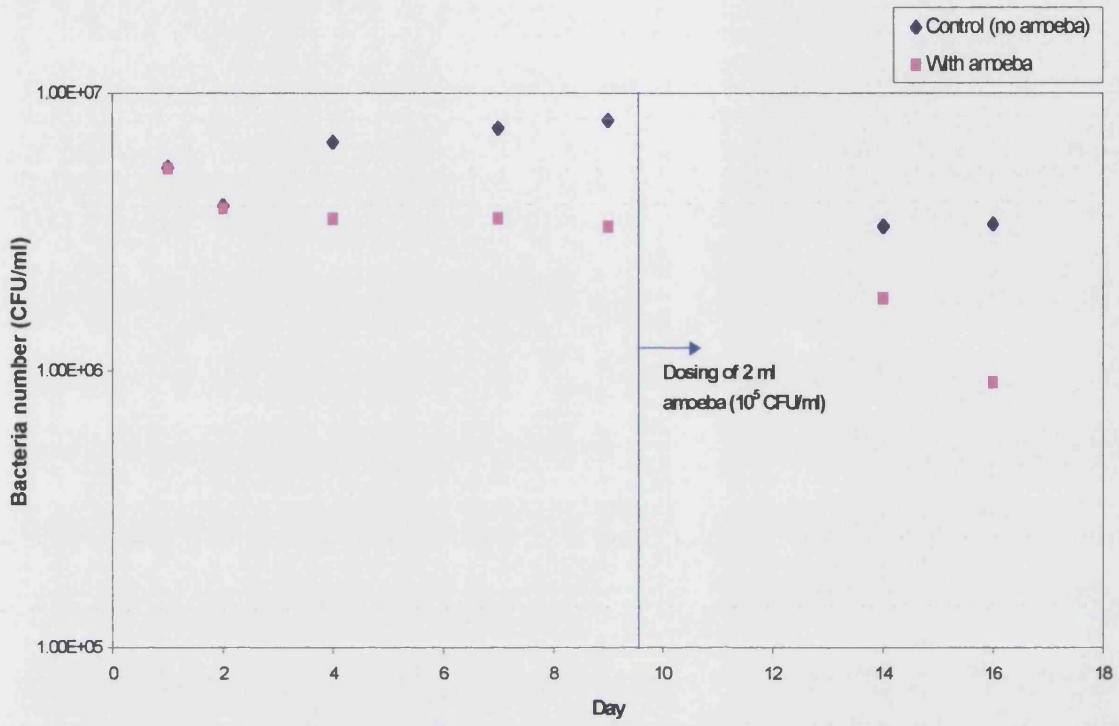


Figure 6.19: Bacteria number from plate count against time (1st amoeba test)

The results of the test showed some reduction in bacteria number with the addition of *Acantyst* amoeba. However, this was not a significant reduction (28 – 38 % compared to the initial CFU on day 1). It was observed from the microscopic examination that the amoeba had encysted after a few days. Hence, on day 9, another dosage of amoeba (2 ml of 10^5 cells/ml) was added. This led to a significant reduction in the bacteria number as observed in Figure 6.19. This could partly be due to the natural lysis of the bacterial cells under starvation, but the comparison with the control flask B showed that there was still 45 – 73 % reduction in CFU. This proved that *Acantyst* amoeba was playing a role in reducing the bacteria number.

Control flask B saw an increase in the bacteria number on the 3rd day. It could be due to the bacteria surviving on their reserve polymers and hence growth was observed. However, after day 9, there was a decrease in the bacteria number in the control flask.

This may have been due to the natural lysis of the bacteria as a result of starvation and exhaustion of their reserve polymers.

Although the test showed success in employing *Acantyst* amoeba in reducing the bacteria number, there were still uncertainties and questions to be asked. The microscopic observation of amoeba encysting after a few days showed that the environment was unfavourable for their survival. This could be due to the scarcity of the viable bacteria coming from MBR 2. Besides, the microscopic examination also showed the coexistence of a type of rotifer and ciliated protozoa. Hence, it was unclear if the sole contribution towards the reduction of bacteria number came alone from the *Acantyst* amoeba. Also, the coexistence of other higher organisms in the system might have been the reason for the amoeba to encyst after a few days.

6.4.2 Second set of experiments

In order to attempt to solve the problem of amoeba encysting in the system, it was proposed that some nutrient PYG supplement (Peptone, Yeast and Glucose) to be added alongside with the amoeba. The *Acantyst* amoeba had been grown in the PYG supplement and it was hoped that the PYG supplement might help to stimulate and sustain the survival of amoeba in the system. One concern with the addition of PYG supplement was that this might also provide a nutrient for the bacteria to grow on. Therefore, another control with bacteria and PYG supplement was set up to observe the effect of PYG on bacterial growth.

6.4.2.1 Materials and Methods

There was a slight modification in the 2nd test, whereby a higher volume of the test sample was used. Four 100 ml volumetric shake flasks were used for the test. Each contained 50 ml of sludge sample (50% dilution with saline) withdrawn from MBR 2. At the start, the following were added to each of the four flasks:

A – 2 ml of 6.3×10^5 cells/ml amoeba + 0.5 ml of saline,

B – 2 ml of 6.3×10^5 cells/ml amoeba + 0.5 ml of PYG supplement (Peptone, 3.75 g/l; Yeast extract, 1.25 g/l; Glucose, 0.18 g/l),

C – control (no amoeba) + 2.5 ml saline,

D – control (no amoeba) + 2 ml saline + 0.5 ml of PYG supplement

The flasks were kept in a water bath at a constant temperature of 20 °C. The pH of the samples in all the flasks was monitored and recorded. Slight air bubbling with a fine air stone provided the oxygen needed for the sludge. The systems were wrapped up to avoid too much light for the amoeba. Everyday 0.1 ml of sample was pipetted onto agar plates for plate count study.

6.4.2.2 Results and Discussion

At the start of the test before the addition of amoeba, the pH of the sludge was 5.98 at 17 °C. Two drops of 40% KOH was added to bring up the pH to 7.08 at 17.1 °C.

The results are tabulated in Table 6.2 and plotted in Figure 6.20. It could be seen from Figure 6.20 that the bacteria number for B, C and D are similar at the beginning of the test (~ 7.75×10^6 CFU/ml). However, the CFU for A was lower than this, at 5.33×10^6 CFU/ml. This may have been due to human error in the plate count study. The next day, growth was observed in all four flasks. On the 3rd day, a drop in the CFU was observed in all four flasks. For the control flasks (C and D), the drop in CFU number may be caused by natural lysis possibly due to exhaustion of food in the systems. It was evident that the addition of PYG supplement did not stimulate the growth of the bacteria as there was a decrease in the CFU of control flask D (with PYG).

Table 6.2: Results for the 2nd amoeba test.

Day	Bacteria number (CFU/ml)				Comparison of A and C (% reduction)	Comparison of B and D (% reduction)
	A (amoeba)	B (amoeba + PYG)	C (control)	D (control + PYG)		
1	5.33 E+06	7.66 E+06	7.44 E+06	7.99 E+06	28.36	4.17
2	7.33 E+06	9.55 E+06	9.99 E+06	9.32 E+06	26.67	-2.38*
3	6.22 E+06	4.55 E+06	5.88 E+06	7.99 E+06	-5.66*	43.06

*Note: * indicates an increase in CFU*

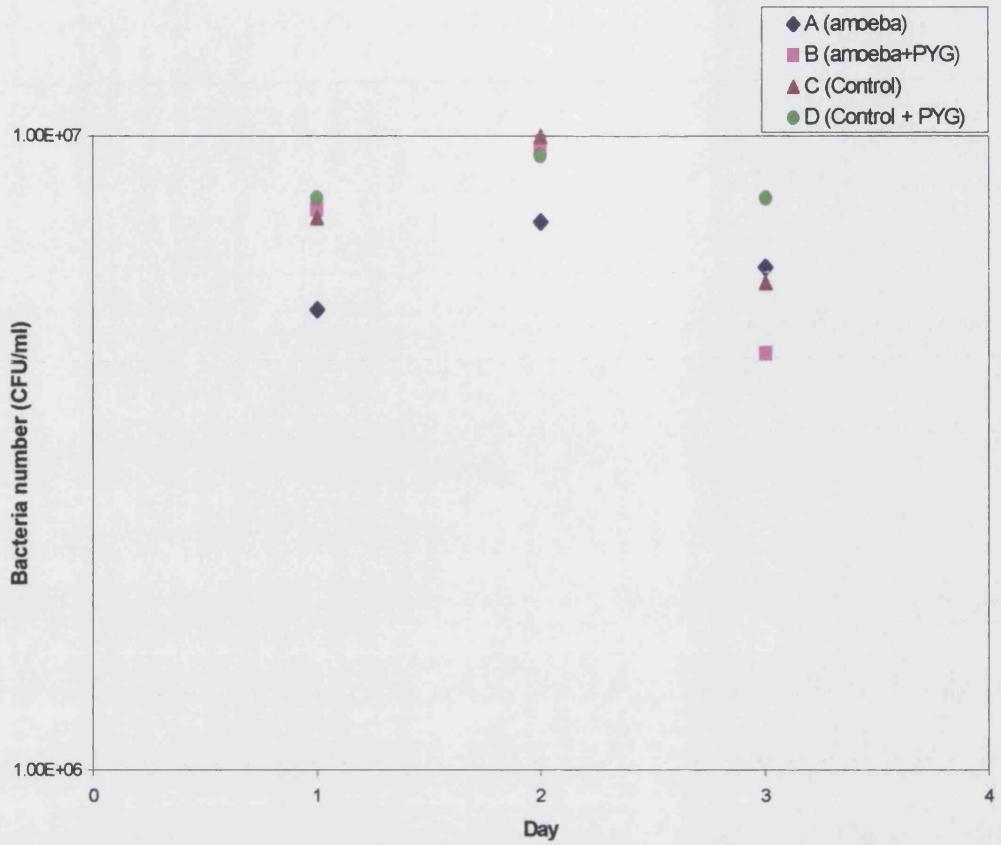


Figure 6.20: Bacteria number from plate count against time (2nd amoeba test)

For A, it could be seen that there was a slight overall growth in the bacteria CFU number. This may be due to the encysting of the amoeba as a result of an unfavourable environment in the sludge. Hence, the bacteria were able to grow on their reserve polymers. However, for flask B (amoeba + PYG supplement), an overall 41 % reduction in bacteria CFU number on day 3 compared to the initial CFU on day 1 (or a 43 % reduction compared to its control flask D) could be observed. It was therefore hypothesized that the addition of PYG supplement may be a possible alternative to prevent the encysting of amoeba under unfavourable conditions (in this case the insufficiency of bacteria to feed on) and stimulate the growth of amoeba in the sludge in order to reduce the bacteria number.

6.4.3 Third set of experiments

A third test attempting to replicate the results in the second sets of experiments was carried out, but without the required results. It was found out that following the start

of the test, from the microscopic examination, that the amoeba had encysted. The pH of the sludge from MBR 2 was very low, at an approximate pH of 4. This may also have been the reason for the encysting of the amoeba, as acidic environment is unfavourable for their growth and survival. In addition, there were no other higher organisms found in the sludge, compared to when the first test was carried out. The pH reduction was due to acidifying as a result of nitrification in the system, as discussed in Chapter 4.

6.5 Conclusions

The microscopic observations show the presence of higher organisms in all the MBRs. However, the protozoan population is not as diverse as it would be expected in real sewage treatment works as synthetic sewage is employed in this work. Ciliates such as colpidium and vorticellae, rotifer (rotaria) and metazoa (nematode) were observed in the MBRs. MBR 1 has a larger amount of protozoan population compared to MBR 2. This is due to a more viable biomass in MBR 1 as MBR 2 is focused on cell lysis and endogenous metabolism.

From the amoeba test, it is concluded that it would be feasible to employ higher organisms in reducing sludge production, as evidenced by the reduction in the bacterial colony forming unit (CFU). Also, the addition of PYG seemed to be able to stimulate or sustain the growth of amoeba under unfavourable conditions, when the bacteria were not numerous. However, the acidic conditions in MBR 2 were not favourable for sustaining the growth of amoeba as shown from the 2nd test. Therefore, the *Acanthyst* amoeba might not be such a suitable agent / prey to be employed. Future studies involving the use of acid-resilient higher organisms might prove to be beneficial. Otherwise, the acidity of MBR 2 could be rectified by the addition of alkali, where the use of *Acanthyst* amoeba or other higher organisms may be advantageous in reducing sludge production.

Chapter 7: Mathematical Model and Cost analysis

Scope of Chapter 7

This chapter outlines the development of a steady state mass balance mathematical model based on Monod kinetics. The results of the model, solved with kinetic parameters found from respirometry (Chapter 5), are compared to the long term steady state experimental results under the same operating conditions. Also, a cost analysis for the two-stage MBR concept is presented and potential cost savings are determined from the preliminary analysis in order to demonstrate the cost feasibility of the two-stage MBR concept.

7.1 Mathematical Model

A simple mathematical model based on steady state mass balance equations is developed to predict the performance of the MBR in terms of effluent quality and sludge yield. This model takes into account of the removal of the organic matter only, excluding the nitrification and denitrification processes, to reduce the complexity of the model.

7.1.1 Schematic Diagram of a 2-Stage MBR System

A schematic diagram of a 2-stage MBR system is shown in Figure 7.1.

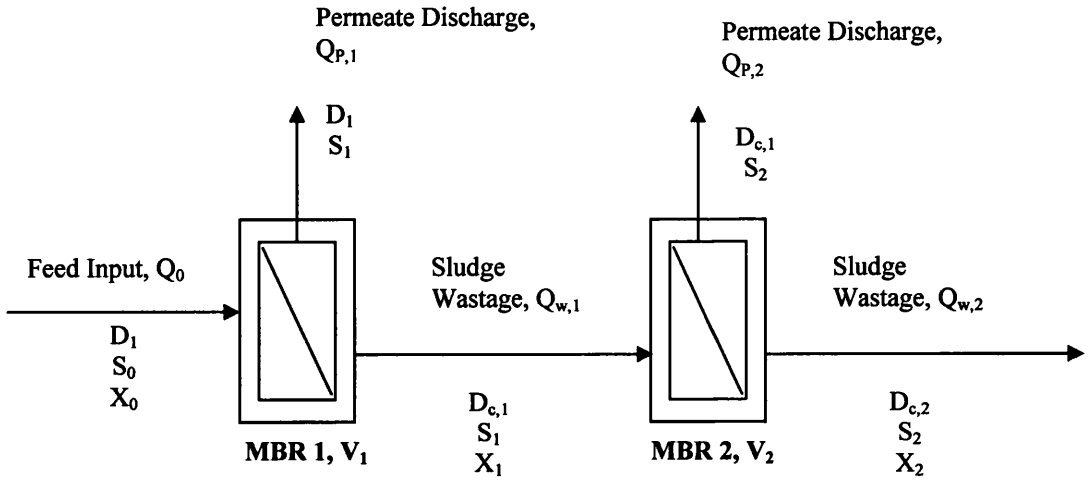


Figure 7.1: Schematic diagram of a two-stage MBR system

7.1.2 Model Assumptions and Development

The following assumptions have been made in order to solve the two-stage mass balance model:

1. All the sludge wasted from the 1st stage (MBR 1) is hydrolysed back to BOD and goes into the 2nd stage (MBR 2) as input feed
2. If all the cells can be oxidised completely in the endogenous respiration process, the ultimate BOD of the cells is equal to 1.42 times the concentration of cells (Metcalf and Eddy, 1991)
3. It is desirable to have a maximum dilution rate (i.e. minimum HRT) and a sludge age that exceeds 10 days (SRT of MBR 1 is 16 d)
4. A dilution rate, $D_1 = 0.17\ h^{-1}$ (based on a 6 h HRT for MBR 1) is used in this model
5. The constraints on this model are:
   a) the maximum expected biomass that can be successfully supported in the aeration tank
   b) the throughput of the reactor
6. The incoming biomass, X_0 is negligible.
7. Sum of outflow is equal to the inflow, i.e. $Q_0 = Q_P + Q_w$
8. There is no biomass in the permeate discharge streams as total retention of biomass is assumed with the use of membrane separation module
9. The steady state oxygen concentration is to be maintained at or above 2 mg/l.
10. With a given membrane surface area, the permeate flow Q_P, can be calculated at a specific permeate flux.

The Monod kinetics (Monod, 1949) has been assumed, so that the following steady state mass balance equations may be derived:

For substrate, S in MBR 1:

Substrate = Inlet S – Outlet S – Substrate utilization rate, r_s

Accumulation

$$V_1 \frac{dS_1}{dt} = Q_0 S_0 - (Q_P + Q_w) S_1 - \frac{\mu_1 X_1 V_1}{Y_{X/S}} \qquad (7.1)$$

For biomass, X in MBR 1:

Biomass = Inlet X – Outlet X + Growth rate, r_x – Endogenous decay rate

Accumulation

$$V_1 \frac{dX_1}{dt} = Q_0 X_0 - Q_w X_1 + \mu_1 X_1 V_1 - k_{d,1} X_1 V_1 \qquad (7.2)$$

The same sets of equations could be written for the steady state substrate, S_2 and biomass, X_2 in MBR 2.

Also the dilution rates of the system are given by the equations below:

$$D = \frac{Q_0}{V_1}; \qquad D_{c,1} = \frac{Q_{w,1}}{V_1}; \qquad D_{c,2} = \frac{Q_{w,2}}{V_2} \qquad (7.3)$$

The specific growth rate is given by the Monod kinetic equation with S as the limiting substrate:

$$\mu_1 = \frac{\mu_m S_1}{K_{S,1} + S_1} \qquad (7.4)$$

Under the assumption of steady state conditions and also considering the above mentioned assumptions, a number of equations (Equations 7.5-7.10) are obtained.

It was found from the mass balances that the specific growth rate, μ_1 is also a function of the cell dilution rate, $D_{c,1}$ and death rate, $k_{d,1}$:

$$\mu_1 = D_{c,1} + k_{d,1} \qquad (7.5)$$

Therefore, the steady state substrate concentration in MBR 1, S_1 can be calculated using the following equation:

$$S_1 = \frac{K_s (D_{c,1} + k_{d,1})}{\mu_m - D_{c,1} - k_{d,1}} \qquad (7.6)$$

The steady state biomass concentration in MBR 1, X_1 is given by:

$$X_1 = \frac{D\ (S_0 - S_1)\ Y_{X/S}\ (K_s + S_1)}{\mu_m S_1} \qquad (7.7)$$

The feed input to MBR 2 is given by the sum of S_1 and the hydrolysed biomass, X_1 into BOD:

$$S_{2,input} = S_1 + 1.42X_1 \qquad (7.8)$$

The steady state substrate concentration in MBR 2, S_2 can be calculated from:

$$S_2 = \frac{K_s(D_{c,2} + k_{d,2})}{\mu_m - D_{c,2} - k_{d,2}} \qquad (7.9)$$

The steady state biomass concentration in MBR 2, X_2 is given by:

$$X_2 = \frac{D_{c,1}\ (S_{2,input} - S_1)\ Y_{X/S}\ (K_s + S_2)}{\mu_m S_2} \qquad (7.10)$$

7.1.3 Results and Discussions

The kinetic parameters and process variables used for both MBR 1 and MBR 2 are tabulated in Tables 7.1 and 7.2.

Table 7.1: Kinetic parameters determined from respirometry studies for MBR 1 and MBR 2 (see Chapter 5).

Kinetic Parameters	MBR 1 (Yield = 0.25)	MBR 1 (Yield = 0.65)	MBR 2 (Yield = 0.13)
μ_m (h^{-1})	$0.018 \pm 0.004^*$	$0.15 \pm 0.011^*$	0.0033
K_s (mg COD/l)	$145 \pm 61^*$	$38.82 \pm 6.3^*$	86
k_d (d^{-1})	0.0084 ± 0.001	0.0084 ± 0.001	$0.015 \pm 0.001^\wedge$

Note: $^*\mu_m$ and K_s determined with yield of MBR 1 = 0.25 or 0.65 g SS / g COD

$^\wedge k_d$ for MBR 2 was determined theoretically with Equation 5.14

Table 7.2: Process variables of MBR 1 and MBR 2 used in the model.

Variables	MBR 1	MBR 2
Feed Input Substrate, S (mg/l)	400	$S_1 + 1.42\ X_1$
Volume, V (l)	3.5	3.5
Membrane Area, A (m^2)	0.12	0.12

As mentioned in Chapter 5, the kinetic parameters determination was complicated by the fact as to which cell / sludge yield should be employed in the determination of the parameters from the batch respirometry studies. From the point of view that the batch respirometry were carried out with higher F/M or initial S_0/X_0 ratio than that for MBR in reality, a higher cell yield of 0.65 should be employed. However, the concern raised by other researchers regarding batch cultivation conditions affecting the proportion between groups of microorganisms from the activated sludge biocenosis by favouring fast growers under higher S_0/X_0 ratios puts a question mark as to the accuracy of the kinetic parameters determined with batch respirometry studies under high F/M or initial S_0/X_0 ratio. Therefore, it was decided that the kinetic parameters determined with the experimental steady state sludge yield of 0.25 for MBR 1 should be employed for the purpose of verification of the mathematical model. Table 7.1 shows the kinetic parameters determined from respirometry studies for MBR 1 and MBR 2 that were used to solve the model. The performance predicted by the model is then compared to the long term experimental steady state performance of MBR 1 and the results are tabulated in Table 7.3.

Table 7.3: Comparison of experimental results of MBR 1 with theoretical prediction of the model solved with kinetic parameters (determined with the experimental sludge yield of 0.25 for MBR 1).

Kinetic parameters	Model prediction, mod	Experimental Results, exp
Permeate COD, S_1 (mg COD/l)	28.2	27.4
MLSS, X_1 (mg/l)	5,378	5,280
Sludge yield, Y_1 (g SS/ g COD)	0.22	0.25

Table 7.4: Comparison of experimental results of MBR 1 with theoretical prediction of the model solved with kinetic parameters (determined with the experimental sludge yield of 0.65 for MBR 1).

Kinetic parameters	Model prediction, mod	Experimental Results, exp
Permeate COD, S_1 (mg COD/l)	0.77	27.4
MLSS, X_1 (mg/l)	14,834	5,280
Sludge yield, Y_1 (g SS/ g COD)	0.57	0.65

The model solved with kinetic parameters determined by respirometry shows the ability to predict realistic results as seen from the relative small differences when compared to the long term steady state experimental results for MBR 1 (see Table 7.3). This has verified the feasibility of the kinetic parameters determined for MBR 1, though their application in a wider context to other MBR systems has yet to be proven.

As for MBR 2, attempts to determine the kinetic parameters with batch respirometry studies was unsuccessful due to the fact that there was no significant growth observed (refer to Chapter 5), which made it impossible to determine growth constants such as μ_m and K_s. This was because MBR 2 did not receive a constant supply of readily degradable organic substrate. MBR 2 received only sludge wasted from MBR 1, which consists of little readily soluble organics as the permeate COD of MBR 1 was very low ($\approx$ 27 mg COD/l). As a result, it was difficult to find a suitable substrate to be employed in the batch respirometry studies to determine the kinetics of MBR 2. One set of results determined from the studies that seems reasonable was tabulated in Table 7.1. Table 7.5 shows a comparison of the results predicted by the model solved with the kinetic parameters determined for MBR 2 with the long term experimental steady state performance of MBR 2.

Table 7.5: Comparison of experimental results of MBR 2 with theoretical prediction of the model solved with kinetic parameters.

Kinetic parameters	Model prediction, mod	Experimental Results, exp
Permeate COD, S_2 (mg COD/l)	34.7	58.8
MLSS, X_2 (mg/l)	4,662	11,770
Sludge yield, Y_2 (g SS/ g COD)	0.06	0.14

Table 7.5 shows a large difference between the results predicted by the model and the long term steady state experimental results for MBR 2. There are a few possible reasons to the inaccuracy of the model in predicting the performance of MBR 2. First of all, the validity of the kinetic parameters determined with batch respirometry studies for MBR 2 has yet to be established. Future work has to be carried out to find the suitable substrate to be employed for the batch respirometry studies for MBR 2. This also leads to the second reason as to why the model deviates from the experimental results. There is still a lack of understanding of the different processes that are actually occurring in MBR 2, especially those at a microbial level (lysis, cryptic growth, predation, etc.). Therefore, Monod kinetics which predicted the performance of MBR 1 may not be applicable to MBR 2, as the metabolism in MBR 2 differs from that in MBR 1. Furthermore, the model itself was too simplistic and was not sufficient to describe what was happening in MBR 2. According to IAWQ Activated Sludge Model No. 2 (Henze *et al.*, 1995), the readily biodegradable substrate, S_S is utilised for growth of heterotrophic biomass, X_H. The biomass, X_H then decay into slowly biodegradable substrate, X_S and inert biomass, X_I. The slowly biodegradable substrate, X_S is subsequently hydrolysed back to readily biodegradable substrate, S_S (the rate limiting step). Figure 7.2 illustrates a simplified flowsheet of components based on ASM 2. However, the developed model in this work did not take into account of the different fractions of biomass (X_H, X_S and X_I). Hence, in order to predict the performance of MBR 2 more accurately, a more complicated and comprehensive model has to be developed. This cannot be achieved until further work to gain a better insight into understanding MBR 2 has been carried out.

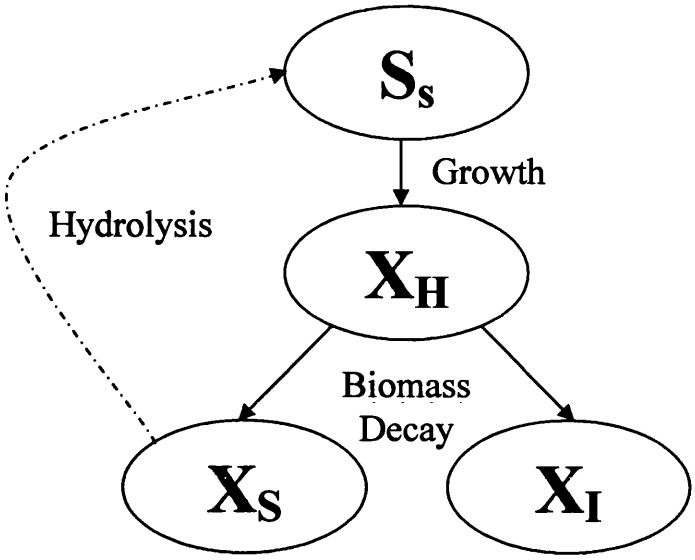


Figure 7.2: Simplified flowsheet of components based on ASM 2.

7.2 Cost feasibility studies of the two-stage MBR system

The experimental findings from this work have proven that the two-stage MBR system has the potential of producing at least 50% less sludge in comparison to a single stage MBR system (refer to Chapter 4). Therefore, the experimentally verified MBR performance data and operating conditions form the basis of the following cost feasibility studies. In this section, the economics of the two-stage MBR system is studied and compared to that of a single stage MBR system, to investigate the potential cost savings resulting from the reduced sludge production. The cost impact from the addition of a second tank for the two-stage MBR concept is also investigated. The economics of the scale-up MBR plants is also calculated based on data from the Kubota MBR plant in Swanage, Dorset, UK. It is the first large scale submerged membrane bioreactor plant of its type in the world, commissioned in July 2000 and operated by Wessex Water Services Limited.

7.2.1 Data from Swanage MBR plant and assumptions for the basis of cost studies

Swanage MBR plant is located in a picturesque bay, with nearby bathing beach, in the South of England. It is designed to treat effluent from a summer population of 28,000. The flow to full treatment of the plant (maximum design flow) is 12,700 m^3/d. For the purpose of the costing, average population equivalence (PE) of 23,000 and an average daily input flow of 6,000 m^3/d are assumed.

There are 132 membrane units in the plant (150 panels of membrane per unit, each with an effective filtration area of 0.8 m^2), giving a total membrane area of 15,840 m^2. The air requirement is 90 m^3/h for each of the membrane units and this gives rise to a total of 11,880 m^3/h for the whole plant. The following scenarios form the basis of the costing calculation:

- Single stage MBR, with a total working volume, V_{total} = 1,650 m^3 in a single tank
- Two-stage MBR, with working volumes for MBR 1, $V_1 = 0.7\ V_{total}$ and for MBR 2, $V_2 = 0.3\ V_{total}$

As mentioned earlier, the MBR performance data and operating conditions have been verified experimentally. Therefore, the results could be used with confidence for the calculation of the volume ratio for the two-stage MBR.

Sludge production, P_X, for the MBRs is given by Equation 7.11 (Metcalf and Eddy, 1991):

$$P_X = Q_W X \qquad (7.11)$$

where P_X = waste sludge production, g MLVSS/d

Q_w = sludge wastage rate, l/d

X = biomass concentration in MLVSS, g/l

As Q_w = V / SRT, where V = working volume of tank and SRT = sludge retention time, Equation 7.11 becomes

$$P_X = \frac{VX}{SRT} \qquad (7.12)$$

Assuming that sludge production of the two-stage MBR is 50% of that of a single MBR 1,

$$P_{X,2\text{-}stage} = \tfrac{1}{2} P_{X,1} \qquad (7.13)$$

Therefore,

$$\frac{V_{total} X_2}{SRT_2} = \frac{1}{2} \frac{V_1 X_1}{SRT_1} \qquad (7.14)$$

The steady state biomass values for MBR 1 and MBR 2 are: X_1 = 4.92 g/l and X_2 = 10.67 g/l. The sludge retention time for MBR 1 and MBR 2 are: SRT_1 = 16 d and SRT_2 = 100 d.

Thus, the calculated volume ratio for the two-stage MBR is: $V_1 = 0.7\ V_{total}$ and $V_2 = 0.3\ V_{total}$.

As V_{total} = 1,650 m^3, the calculated volumes for MBR 1 and MBR 2 are: V_1 = 1,155m^3 and V_2 = 495 m^3.

7.2.2 Capital Cost Estimate for single stage MBR and two-stage MBR systems

The basic functional units included in the costing are the aeration basins (where the membrane will be submerged into, to make up the MBR), coarse bubbles air diffuser, membranes and administration and shop buildings. The construction costs of the different functional units are estimated from the individual unit cost function curves published in the Handbook of Biological Wastewater Treatment (Benjes, 1980). The cost functions curves are estimated construction cost (in Jan 1979) plotted against either the volume of the tanks, the air requirement or the average plant capacity. Based on the design data from Swanage MBR plant and the scenarios as stated above, the construction costs are estimated from these cost function curves.

Cost of flat sheet Kubota membrane is estimated at £80/m^2 or \$125/m^2 in year 2000 (Gander *et al.*, 2000b). However, according to Churchouse and Wildgoose (1999), the projected membrane panels cost has reduced over the years due to the increase in projected membrane life and the scale up of membrane manufacture. Therefore, the cost of membrane is estimated at \$40/ m^2 (for large scale application, where membrane area > 10,000 m^2) for the cost analysis (Arnot, 2003). The membranes required for the two-stage MBR follows the volume ratio for the two stages; whereby 70% of the total membrane area is allocated to MBR 1 and 30% of it to MBR 2. This is also the case for the air requirement for the two stages.

Site work is estimated at 14 % of the total estimated construction cost. Other aspects that make up the total capital cost estimate include engineering, legal, fiscal and administrative at 16.5 %. The land requirement was estimated to be 14 acres from the function curves based on the plant capacity (average daily flow of 6,000 m^3/d) and calculated at $2,500 per acre. Interest during construction is estimated at 8 % and inflation rate to June 2004 when the project will be completed is also estimated at 8 %. These sums up the total project planning cost / capital cost estimate. The cost function curves are estimated for 1979 and then inflated to current costs in June 2003 with the ENR Building Cost Indexes with the following equation:

$$\text{Current cost estimate} = \text{Jan. 1979 cost estimate} \times \frac{current\ index\ value}{Jan.1979\ index\ value} \qquad (7.15)$$

Table 7.6 shows the comparison of the capital cost estimate for single stage MBR and two-stage MBR systems.

Table 7.6 Capital Cost Estimate for single stage MBR and two-stage MBR systems (Benjes, 1980).

Functional Units	Single state MBR		Two-stage MBR	
	Jan 1979	Jun 2003*	Jan 1979	Jun 2003*
Aeration basin	$200,000	$383,220	$250,000	$479,026
Air diffuser (coarse bubbles)	$110,000	$210,771	$121,000	$231,848
Membrane cost		$633,600		$633,600
Administration and shop buildings	$50,000	$95,805	$50,000	$95,805
Subtotal (estimated construction cost)		$1,323,397		$1,440,279
Site work @ 14% of estimated construction cost		£185,276		$201,639
Subtotal		$1,508,672		$1,641,918
Engineering, legal, fiscal, administrative @ 16.5%		$248,931		$270,916
Land @ 14 acres, $2,500 / acre		$35,000		$35,000
Subtotal		$1,792,603		$1,947,835
Interest during construction @ 8%		$143,408		$155,827
Total Estimated Project Cost		$1,936,012		$2,103,661
Inflation to June 2004 (estimate 8%)		$154,881		$168,293
Total Project Planning Cost		$2,090,892		$2,271,954

*Note: *Cost estimates in June 2003 updated with ENR Building Cost Indexes (June 2003 = 3677 and Annual Average 1979 = 1919, base 1913 = 100) (Source: http://enr.construction.com/features/conEco/costIndexes/)*

7.2.3 Operating and Maintenance (O&M) Cost Estimate for single stage MBR and two-stage MBR systems

The operating and maintenance (O&M) costs are estimated for single stage MBR and two-stage MBR systems. The main contribution to the O&M cost is the energy consumption generated from aeration requirement and the sludge disposal cost. Labour is also included in the O&M cost estimate to give a more complete picture. The total aeration requirement for the MBR plant is 11,880 m^3/h. Based on the aeration requirement, the O&M cost are estimated from the cost function curves for diffused aeration system, whereby annual payroll in terms of man-hours and annual electrical energy or power are plotted against the air flow (Benjes, 1980). Unit costs reported for the cost function curves are \$9/h for labour (including overhead and fringes) and \$0.03/kWh for power in Jan 1979. The labour cost for June 2003 is found to be \$24.50/h for utilities sector from the US Bureau of Labour Statistics website (source: http://www.bls.gov/data/home.htm). The current cost of power is estimated to be \$0.10/kWh (Brown, 2003). The annual payroll of 5167 hours also includes the estimated manpower needed for the operation of the MBR plant. Energy consumption of 3,250,000 kWh is needed to provide the total aeration requirement for the whole MBR plant. Sludge production is estimated to be 42 kg dry solids/d for a MBR plant catering for 800 – 1,200 PE (source: http://www.mbrtech.com/). Therefore, for Swanage MBR plant with an average of 23,000 PE, sludge production of 1200 kg DS/d is estimated for single stage MBR and 600 kg DS/d is estimated for two-stage MBR (based on a 50% sludge reduction for two-stage MBR compared to single stage MBR found from this work). The sludge disposal cost of £175/tonne dry solids or \$280.52/tonne dry solids is assumed (Pearce, 2002). Table 7.7 shows the comparison of the O&M cost estimate for single stage MBR and two-stage MBR systems.

Table 7.7 Operating and Maintenance (O&M) Cost Estimate for single stage MBR and two-stage MBR systems (Benjes, 1980).

Cost Parameters	Single state MBR		Two-stage MBR	
	Jan 1979	Jun 2003	Jan 1979	Jun 2003
Air diffuser @ 11,880 m^3/h				
Annual payroll (5,167 h) @ $9/h (1979) and $24.50/h (2003)	$46,503	$126,592	$46,503	$126,592
Power (3,250,000 kWh) @ $0.03/kWh (1979) and $0.10/kWh (2003)	$97,500	$325,000	$97,500	$325,000
Annual sludge production (tonne DS/yr)	438		219	
Annual sludge disposal cost @ £175 /tonne DS or $280.52 /tonne DS		$122,868		$61,434
Total O&M Cost		$574,460		$513,026

Note: 1. Exchange rate £1 → $1.60298 (1 Aug 2003). Source: http://www.xe.com/ucc/

2.Sludge production of 42 kg DS/d is assumed for 800 – 1,200 PE (Source: http://www.mbrtech.com/). Therefore, with Swanage (23,000PE), sludge production of 1200 kg DS/d is assumed for single stage MBR and 600 kg DS/d is assumed for two-stage MBR (based on experimental findings of 50% sludge reduction for two-stage MBR compared to single stage MBR).

7.2.4 Results and discussions

Table 7.8 shows a summary of the capital and O&M cost estimates for single stage MBR and two-stage MBR. The total capital investment cost for a single stage MBR is $2.09 million, whereas for the two-stage MBR, it is $2.27 million. For the two-stage MBR, the total capital investment cost is higher due to an additional second tank. However, the total annual O&M cost is lower for the two-stage MBR ($0.51 million) compared to the single stage MBR system ($0.57 million) due to the cost savings obtained from the 50% less sludge production. Straight-line depreciation method is used to calculate the annual depreciation (d) for the MBR plant and is given by the following equation (Peters and Timmerhaus, 1991):

$$d = \frac{V - V_S}{n} \qquad (7.16)$$

where d = annual depreciation, $/year

V = original value of the plant at start of the service-life period, completely installed and ready for use, $

V_S = salvage value of the plant at end of service life, $

n = service life of plant, years

The working life of the MBR plant is estimated to be 25 years and the salvage value is estimated at 10% of the initial value of the plant.

The total annual expenditure is the sum of annual O&M cost and annual depreciation (d). Assuming the MBR plant is treating an average daily flow of 6,000 m^3/d, this gives rise to a total annual processing rate of 2,190,000 m^3/yr. The unit processing cost for the MBR treatment plant can therefore be calculated as:

$$\text{Unit processing cost, } \$/m^3 = \frac{\text{Annual expenditure, } \$/yr}{\text{Annual processing rate, } m^3/yr} \qquad (7.13)$$

The overall unit processing cost for two-stage MBR ($0.272/$m^3$) is lower compared to a single stage MBR ($0.297/$m^3$). The cost feasibility studies shows that the two-stage MBR system offers a saving of $54,916 in the total annual expenditure compared to a single stage MBR. Furthermore, two-stage MBR system offers a 50% less sludge production (219 tonne/yr) compared to the single stage MBR (438 tonne/yr). This leads to a lesser environmental impact from sludge disposal.

Table 7.8 Summary of cost estimate for single stage MBR and two-stage MBR.

Cost Estimate or parameters (Units)	Single stage MBR	Two-stage MBR
Total Capital Investment ($)	$2,090,892	$2,271,954
Annual Operating and Maintenance (O&M) cost ($/yr)	$574,460	$513,026
Annual depreciation, d ($/yr)	$75,272	$81,790
Total annual expenditure ($/yr)	$649,732	$594,816
Annual processing rate (m^3/yr)	2,190,000	2,190,000
Unit processing cost ($/$m^3$)	$0.297	$0.272
Savings in total annual expenditure ($/yr)	-	$54,916
Environmental Impact – Annual sludge production (tonne/yr)	438	219

Note: 1. Straight line depreciation is used to calculate the annual depreciation for the MBR treatment plant over a working life of 25 years.

2. An average daily flow / processing rate of 6,000 m^3/d is assumed for the MBR treatment plant.

3. A salvage value of plant at end of service life, V_s=10% of the initial value of plant (total capital investment) is assumed.

4. Savings in annual O&M cost for two-stage MBR is calculated against single stage MBR (due to the 50% less sludge production).

5. The environmental impact is shown by the annual sludge production of the plants. Two-stage MBR causes half of the impact on the environment due to the 50% less sludge production compared to single stage MBR..

7.3 Conclusion

A mathematical model based on Monod kinetics and steady state mass balance for the MBRs is developed to predict the effluent quality and sludge production. The model solved with kinetic parameters determined by respirometry for MBR 1, shows the ability to predict realistic results when compared to the long term steady state experimental results for MBR 1 (see Table 7.3). This is however not the case for MBR 2. Due to the complexity of the metabolism occurring in MBR 2 and the difficulty in determining the kinetic parameters for MBR 2 from respirometry studies, the model was not sufficient in predicting the performance of MBR 2. In addition, Monod kinetics may not be applicable for MBR 2, since endogenous respiration or cell lysis dominates in MBR 2 rather than cell growth. Therefore, more work needs to be carried out to understand the processes in MBR 2 and a more comprehensive model could then be developed to predict the performance of MBR 2.

The cost feasibility studies show that the two-stage MBR offers potential cost savings ($54,916/yr) and less environmental impact due to the 50 - 60% less sludge production in comparison to a single stage MBR system, as determined from the experimental findings of this work. The studies also show that the addition of a second stage did not result in a big increase in the capital cost. Moreover, the overall unit processing cost of treating the wastewater for two-stage MBR ($0.272/$m^3$) is lower compared to a single stage MBR ($0.297/$m^3$). For future work, it is proposed that with a two-stage MBR system, there is the opportunity to manipulate the volume ratio of the two stages in order to achieve a better cost savings and lesser sludge production.

Chapter 8: Conclusions and Recommendations

Scope of Chapter 8

This chapter summarises the conclusions obtained from the results chapters of this work (Chapter 4, 5, 6 and 7) and further outlines the recommendations for future work.

8.1 Conclusions for Chapter 4 Performance of the MBRs

Chapter 4 examines the performance of the two-stage MBR and the promising results validate the experimental feasibility of the two-stage MBR concept.

1. Permeate of MBR 1 meets the stringent discharge limits with approximate BOD_5 and COD values of 4 mg BOD_5/l and 27 mg COD/l.
2. The excellent BOD_5 and COD removal rates of MBR 1 (98% for BOD_5 reduction and 91 % for COD reduction) are unrivalled by the conventional activated sludge (AS) process.
3. Two-stage MBR gives a much lower sludge yield of 0.13 kg MLSS/kg BOD, in comparison to other wastewater treatment processes such as BAF, trickling filter, conventional AS.
4. The two-stage MBR offers an overall sludge reduction of 62.2% in comparison to the single MBR 1.
5. The two-stage MBR configuration has a lower volumetric sludge production rate compared to the single stage MBR 1, which leads to substantial economic savings on the overall sludge disposal and treatment cost.
6. The volumetric COD removal rate of MBR 1 was overall 70 % higher than MBR 2. These results are in accordance to the proposed concept for the two-stage MBR configuration, where the first stage is focused on high COD / BOD removal rate and the second stage is focused on reduced sludge production rate.
7. MBR 1 has a higher SOUR value than MBR 2. This indicates that MBR 1 has a higher biological activity than MBR 2, due to the biomass being more viable and actively metabolising the synthetic feed in MBR 1; while MBR 2 is more focused on cell lysis and endogenous respiration.

8. Permeate of MBR 2 also shows excellent BOD_5 and COD values of approximately 12 mg BOD_5/l and 59 mg COD/l, which are well below the discharge limits.
9. MBR 2 shows a gradual pH drop due to nitrification and it experienced disturbance from alga bloom. This led to the start-up of a smaller scale new MBR 2. The new MBR 2 also shows a gradual pH drop since start-up.
10. The nitrogen tests show that there was nitrification in the MBRs. However, the nitrification from ammonia to nitrate was inhibited in MBR 1, as shown by the build up of intermediate nitrite concentration in permeate of MBR 1.
11. For both the original and the new MBR 2, there was a high concentration of ammonia present in the permeate due to cell lysis into organic nitrogen matter. The high nitrate and low nitrite concentration shows a high nitrification activity.
12. The nitrogen concentration in the permeate streams of MBRs did not meet the discharge limits. The nitrogen load in the synthetic feed recipe is higher in organic form (protein present in peptone and meat extract) which is unstable and subject to further decomposition, compared to a real wastewater effluent that has higher inorganic nitrogen content. Therefore, the degradation of the organic nitrogen by the bacteria releases more ammonia nitrogen, which is then converted into nitrite and nitrate, leading to higher nitrogen concentrations in the permeate streams. However, if real sewage is employed in the MBR, the performance of the MBR would be expected to be improved.
13. Nitrification could be inhibited due to limited oxygen concentrations and non-optimal pH conditions. Therefore, it is necessary to ensure that dissolved oxygen concentration is not limiting and alkalinity control is employed to enhance nitrification. Alternative process design (including a separate nitrification / denitrification stage) and a different operational parameter (longer SRT) would possibly improve the quality.
14. The fouling tests show that the critical flux determined for both MBR 1 and MBR 2 was 10 LPM. However, the TMP against flux graphs show that the MBRs do not exhibit the same fouling characteristics, as seen from the different hysterisis effects, even though the tests were carried out at similar MLVSS concentrations. The sludge in MBR 2 showed a tendency for a higher degree of fouling when the critical flux was exceeded. The critical flux information for the MBRs is crucial in

designing the operation parameters to allow the MBRs to operate for a long period of time with minimum fouling.

8.2 Conclusions for Chapter 5 Respirometry as a tool for determination of kinetic parameters for MBR 1 and MBR 2

Chapter 5 outlines the kinetics results obtained from batch resprometric studies for MBR 1 and MBR 2.

1) For MBR 1, the batch respirometric studies were carried out at an F/M or S_0/X_0 ratio between 0.014 and 1.775. The kinetic parameters, μ_m and K_s were obtained for MBR 1 based either on the experimental yield from the continuous operation of MBR 1 (0.25 g SS/ g COD) or the theoretical reported yield of 0.65 g SS/ g COD.
2) The curve fit analysis showed higher standard errors (S) and lower correlation coefficients when a higher yield (Y = 0.65) was used. However, μ_m and K_s calculated with Y = 0.65 were closer to the reported literature values. This was because in a batch respirometer, the biomass is subjected to an environment rich in substrate in order to promote exponential growth to determine the growth constants, μ_m and K_s. Therefore, a higher yield will better correspond to the growth behaviour of the biomass in a batch respirometer.
3) μ_m estimated for MBR 1 has a mean value of 3.6 d^{-1} (assuming a yield of 0.65 and on the basis of COD for the calculation in the batch respirometric studies), which is reasonable when compared to the work of Sözen *et al.* (1998) on the basis of initial S_0/X_0 ratio. They reported μ_H values in the range of 3.4 – 6.5 d^{-1} for domestic sewage, carried out with S_0/X_0 ratios between 0.43 and 0.86. The μ_m from this work also compares well with other reported literature values.
4) The mean K_s determined for MBR 1 (38.8 mg COD/l) is well in agreement with the reported value of Metcalf and Eddy (1991), with the range of K_s for the activated sludge process for domestic wastewater to be between 15 – 70 mg COD/l, with a typical value of 40 mg COD/l.
5) There are concerns raised about the use of batch respirometery (Novák *et al.*, 1994a), as during batch cultivation, the microorganisms are exposed to different living conditions (much higher F/M ratio) than in continuously operated systems.

Therefore, the batch cultivation methods for the determination of kinetic and stoichiometric parameters for the purposes of mathematical modelling of biological wastewater treatment processes should be applied with caution.

6) There was difficulty in finding a suitable substrate for MBR 2 to be employed in the batch respirometric studies. There was no obvious growth to be observed and it was not possible to determine the growth kinetic parameters such as μ_m and K_s. The metabolism of the microbial population in MBR 2 was proposed to be different to that of MBR 1, i.e. in a state of lysing / endogenous respiration and starvation rather than growing as in MBR 1.
7) When sludge from MBR 1 was sonicated and its lysis product used as a substrate, μ_m and K_s were found to be 0.0033 h^{-1} and 86.03 mg COD/l. However, these results were not repeatable; therefore further future work is necessary to verify their validity.
8) The k_d or b_H values reported in the literature, either assumed for mathematical modelling or experimentally determined, shows wide variability. The b_H values varied between 0.077 – 0.25 d^{-1} under different conditions such as sludge age, temperature, wastewater characteristics, etc.
9) Metcalf and Eddy (1991) reported k_d for the activated sludge process for domestic wastewater to be between 0.025 – 0.075 d^{-1}, with a typical value of 0.06 d^{-1}. The average k_d for MBR 1 determined between 60 to 120 h (0.01 d^{-1}) is much smaller compared to the reported values, while the average k_d for MBR 1 determined between 8 to 20 h (0.05 d^{-1}) is more in agreement with the reported values.
10) For MBR 2, k_d was calculated either from the steady state mass balance model performed around MBR 2, given by Equation 5.14, or determined experimentally from batch respirometry. The theoretical calculated k_d (average of 0.015 d^{-1}) showed more consistent results while the experimental k_d (average of 0.021 d^{-1}) showed very scattered results.
11) In reference to 10), nevertheless, Equation 5.14 defined the endogenous respiration coefficient as a lumped together-simplified value representing a decrease in cell mass (Metcalf and Eddy, 1991), incorporating many different mechanisms (e.g. maintenance, cell lysis, cryptic growth, predation, etc.) that are still not well understood.

12) The aim for future studies should be focused on developing more reliable and rapid methods for kinetic and stoichiometric parameter determination. In addition, the establishment of a more standardised and refined protocol for batch respirometric studies will ensure better consistency and comparability of the kinetic parameters to be determined by different researchers in the future.

8.3 Conclusions for Chapter 6 Microscopic Observations

Chapter 6 shows the microscopic pictures of the cultures in the MBRs and outlines the sludge reduction experimental results with *Acanthyst* amoeba on sludge from the original MBR 2.

1) The microscopic observations show the presence of higher organisms in MBR 1, original MBR 2 and the new MBR 2. The protozoan population is not as diverse as it would be expected in real sewage treatment works as synthetic sewage is employed in this work.
2) Ciliates such as colpidium and vorticellae, rotifer (rotaria) and metazoa (nematode) were observed in the MBRs. MBR 1 has a larger amount of protozoan population compared to MBR 2. This is due to a more viable biomass in MBR 1 as MBR 2 is focused on cell lysis and endogenous metabolism.
3) Ex-situ sludge reduction experiments with manipulation of higher organism (*Acanthyst* amoeba) were carried out on sludge from original MBR 2. The results from the 1st set of experiments show a reduction in the bacterial colony forming unit (CFU) with the addition of amoeba compared to the control.
4) However, the amoeba encysted after a few days in the test flask, showing that the environment was unfavourable for their survival. This could be due to the scarcity of the viable bacteria coming from MBR 2 and possibly the coexistence of other higher organisms (rotifers and ciliates).
5) A 2nd set of experiments were carried out with the supplement of PYG (peptone, yeast extract and glucose) to stimulate the growth of amoeba. The results show an overall reduction in bacterial number. Therefore, the addition of PYG supplement may be a possible alternative to prevent the encysting of amoeba under unfavourable conditions.
6) The 3rd set of experiments was unsuccessful due to the encysting of amoeba. It was found out that the sludge of MBR 2 had a very low pH of 4 (due to

nitrification) and this subsequently led to the encysting of the amoeba as the acidic environment was unfavourable for their growth and survival. At the same time, no other higher organism was observed in MBR 2 due to the acidity in MBR 2.

7) The manipulation of higher organism as a sludge reduction strategy is feasible as shown by the many researchers with monoculture (see Chapter 2). However, the experiments carried out in this work were on a mixed culture, with the coexistence of other higher organisms. Therefore, it is more difficult to quantify the effect *Acanthyst* amoeba has on the overall sludge reduction.

8.4 Conclusions for Chapter 7 Mathematical model and Cost analysis

Chapter 7 outlines the steady state mathematical model and the predicted results when solved with the kinetic parameters determined from respirometry. Cost analysis of the two-stage MBR concept shows potential savings, which validates the economical feasibility to the concept.

1) The purpose of the mathematical model is to predict the performance of the MBR in terms of effluent quality and sludge yield. When solved with kinetic parameters determined with Y = 0.25, the model gives a better prediction of the performance of MBR 1, with small differences compared to the experimental results. However, this is not the case when the model is solved with kinetic parameters determined with Y = 0.65.
2) The model was unable to predict the performance of MBR 2 when solved with the only set of kinetic parameters determined from respirometry for MBR 2. It is thought that the Monod kinetics may not be applicable to MBR 2 as lysis and endogenous are more dominant than growth. The model is too simplistic and not sufficient to describe what is happening in MBR 2.
3) The cost analysis compares the cost of two-stage MBR to a single stage MBR. The results show that the addition of a second stage did not result in a big increase in the capital cost.
4) The two-stage MBR offers a saving of $54,916 in the total annual expenditure and less environmental impact due to the 50 - 60% less sludge production in comparison to a single stage MBR system.

5) The overall unit processing cost of treating the wastewater for two-stage MBR ($0.272/m^3) is lower compared to a single stage MBR ($0.297/m^3).

8.5 Recommendations

1) Future work could be carried out with real sewage to investigate the performance of the two-stage MBR at different operating parameters (such as SRT and HRT). This will allow the determination of the optimal operating parameters for MBR 1 and MBR 2 to ensure maximal organic removal in the 1st stage and minimal sludge production in the 2nd stage.
2) The issues with the high nitrogen concentrations in the permeate streams have to be looked into. Alternative process design (including a separate nitrification / denitrification stage) could improve the situation. A different operational parameter (longer SRT) would allow longer contact time of the nitrogen with the nitrifiers.
3) As the microorganims are subjected to different living conditions (much higher F/M ratio) during batch respirometry studies than in continuously operated systems, further work needs to be carried out to validate the accuracy of the kinetic parameters determined from this work. Continuous in-situ respirometry could be considered for obtaining the kinetics of the MBRs.
4) The manipulation of higher organisms is promising in reducing the sludge production in the 2nd stage. The process parameters could be optimized to ensure a healthy and diverse protozoan and metazoan population in the 2nd stage. The issues with the pH drop could be corrected by the addition of alkali.
5) Other sludge reduction strategies as mentioned in Chapter 2 (such as the addition of chemical uncouplers, OSA, ozonation, chlorination, etc.) could be employed in the 2nd stage of MBR to further minimise the sludge production.
6) Anaerobic operation instead of aerobic operation could be considered for MBR 2. Without the need for aeration, the operational cost could be reduced. Furthermore, the sludge is highly stabilized and methane gas is produced, which can be used as a profitable end product.
7) More understanding of the different processes (lysis, endogenous respiration, cryptic growth, etc.) occurring in MBR 2 at a microbial level should help in the development of a more suitable model.

8) The model for MBR 1 could be improved by adding processes such as nitrification and taking into account of the different substrates and forms of biomass (readily biodegradable substrate, slowly biodegradable substrate, heterotrophic biomass, autrotrophic biomass, etc.).
9) With a more complete model to describe both MBR 1 and MBR 2, the volume ratio of the two stages could be manipulated to find the optimum design parameters for the two-stage MBR. This will ensure maximal cost-savings and optimal performance of the two-stage MBR.

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